

Scientists don't move

DESCRIBING the British scientific system as positively arthritic, Sir Hermann Bondi introduced a report last week on "Interchange of Scientists" (available free from the Civil Service Department) which is a cautious first step towards easing the joints. It is small comfort that if anything Italy and Germany are even more fixed in their ways.

The report is necessary reading for anyone who worries that the British scientific scene is not so free-flowing and vigorous as its American counterpart, although most who feel that way (and clearly Sir Hermann's Task Force did) must hope that there is more to come and that the group will stay together even just as a continual stimulus to the body scientific and arthritic.

Mobility in the United States is much admired by those who live a trans-Atlantic existence, and with good cause. Scientists and engineers move regularly in pursuit either of greater job satisfaction or more dollars, and few would hazard a guess that they would be doing the same job in five years time. Movement into and out of the civil service, particularly to spend a time in Washington administering a programme they understand well, is something which many excellent scientists do and which gets them much credit in the community. But then 'the community' in the United States much more naturally includes industry and the civil service as well as the universities. Further there is a much greater freedom, it seems, to pay competitive salaries and in particular not to expect a bright young man to wait until he is forty before he begins to profit financially by being a scientist. Thus the problem with stimulating mobility in Britain is ultimately not so much one of improving administrative arrangements and making the physical move less disagreeable as of changing ways of thinking.

The central proposal of the task force (which the government has already promised to implement) is that within the civil service a small unit should be established to facilitate secondment between the civil service on the one hand and industry and universities on the other. Flow should be in both directions. The third side of the triangle—movement between industry and universities—could not be handled by the same unit, but there is an obvious need for an organisation to stimulate interchanges there too. The ever-cautious scientist, scared of venturing into the unknown, will generally be urged out of his shell by the existence of a return ticket to his first employer after a period of, say, two to four years.

The apparent obstacles to mobility which the task force report make stunning reading. The difficulty of moving house, problems with pensions (the university scheme is a particularly difficult one from which to transfer), inadequate allowances for transfer—all testify to feeble-mindedness among scientists and a lack of social responsiveness from employers. It is scarcely believable

that housing is sufficient of an obstacle that the task force has to recommend that "whenever possible, interchanges should be within the same geographical area to avoid moving house". Clearly a spirit of boldness in pushing back intellectual frontiers does not necessarily extend to much else. Not that employers are blameless—"to a large extent, those taking up permanent posts either in the civil service, or elsewhere, are not paid removal expenses". This medieval attitude must surely go.

One of the problems with stimulating mobility is that the potential mover must perceive other areas as attractive. This most certainly is not true at present, indeed the low regard that the three 'wings' of science often hold for each other must often dampen the enthusiasm of even the most willing mover. Furthermore the restraints on recruitment that the civil service has imposed on it ensured that in 1972, for instance, the Principal Scientific Officer grade, which numbers 2,000, comprised only ten who had been recruited from outside the service during the year. And all the signs are that mobility is on the decline. In 1958 half of all university chemists had outside experience. Now less than a third do, and one department of 33 has nobody with external experience!

There is no simple solution to this deplorable situation. The new unit will at least allow a few more temporary exchanges. But ultimately the whole question of the deployment of scientific manpower and the narrowness of institutional and individual attitudes needs penetrating examination. Maybe somewhere along that route the idea of allowing scientists to keep a year of their education in abeyance until their thirties would merit an airing. And it would be good to see more practising scientists in Whitehall.

100 years ago



THE tenacity of life of popular errors is well exhibited in the following extract from the *Californian Horticulturist* :—
 "The influence of forests in drawing moisture from the heavens may be seen from the experience of San Diego, California. Previous to 1863 there was yearly a rainy season, which made the soil nourishing and productive. In 1863 a destructive fire swept over the greater part of the country, destroying the forest and blackening the hills. Since then there has been no rainy season at San Diego." When will public writers learn that forests influence the climate by drawing water, not from the air, but from the soil?

From *Nature*, 10, 253, July 30, 1874.



As a result of the Water Act, 1973, river management, water supply and sewage disposal in Britain have now been integrated under the control of new regional water authorities. This is clearly a desirable move which will make planning and coordination easier within the regions. But of course there are still larger issues which must be settled on a national basis, at government level or by cooperation between regional authorities. To assist with these, a Central Water Planning Unit remains as a rump of the old Water Resources Board, under the auspices of the Department of the Environment (DoE), and a new Water Research Centre has been established by amalgamating the Water Research Association laboratories, the Water Pollution Research Laboratory, most of the Technology Division of the Water Resources Board and part of the Development Division of the Directorate General Water Engineering of the DoE.

As far as the study of long term trends is concerned, the CWPU is concerned directly with forecasting demand, and not with predicting how the supply of water (as rainfall) might vary. But the experience of the past three years, which have been very dry by established standards in Britain, has encouraged an awareness of the question of climatic change, and some steps are being taken to look into this problem.

This new weather pattern is seen by climatologists as part of a global trend which is causing droughts in parts of Africa and has also been responsible for bad harvests in Canada and the USSR. For Britain, the implications are that longer dry spells will be broken by occasional very heavy storms. This has serious repercussions for the farmer, but from the point of view of the CWPU reservoirs can be filled up just as conveniently in one or two great cloudbursts as by weeks of standard British drizzle.

The CWPU itself is situated on the banks of the River Thames at Reading, and members of the Unit (then part of the Water Resources Board) were treated to the sight of the river flooding its banks as recently as January 1974; the same high rainfall which produced that rise in the river helped to stock up the surface reservoirs. But the problems of replenishment of underground aquifers cannot be overcome so easily. Some 25 to 30% of the public water supply is derived from underground sources, and in periods of drought what precipitation there is tends to be absorbed in the surface layers of the ground, or runs off as floodwater, without having much effect on the aquifers; 10% less rainfall over a year can result in as much as a 50% drop in aquifer recharge.

Whither water?

Water services in Britain have recently been reorganised, with the Water Resources Board ceasing to exist on April 1, 1974. John Gribbin discusses future plans for water resources in the light of the last annual report of the Board and the growing awareness of the possible importance of climatic change.

This is where the possibility of longer term changes in rainfall becomes important.

The average rainfall of England and Wales has declined over the past five years; but is this a continuing trend, or is it likely to reverse in the immediate future? It is too early yet for panic measures on the strength of such evidence alone, although it is perhaps worth noting that Professor Hubert Lamb has commented that the first 50 years of this century, which are accepted as the climatic normal, were in

sources Board could, if it so wished, use any part of its research funds in support of climatic research. But now any proposal for extending the present investigation (or, indeed, starting any new project) must first go to the DoE before filtering back down through the CWPU or the Water Research Centre. A spokesman for the DoE confirmed recently that proposals for future research along these lines have been discussed with Professor Lamb, who heads the Climatic Research Unit, and that "the proposals are now under consideration by the Department". Meanwhile, the planners have plenty on their plate in working out how to distribute the rainfall we do get, without worrying too much about what might happen to that rainfall in future.

The way the CWPU would like to see things develop is towards what might loosely be termed a 'national water grid'. This would involve transfers from region to region—such as from the Severn to the Thames—and extending the present system of river regulation. Again, however, this depends on how things develop as the new regional authorities feel their way. There is nothing to stop a region opting for

Table 1 Rainfall over England and Wales, for year ended September 30, 1973

Month	Monthly rainfall (mm)	1916-50 Standard average (mm)	Percentage of average
1972			
October	32	92	35
November	99	95	104
December	104	88	118
1973			
January	44	92	48
February	40	66	61
March	24	57	42
Winter	343	490	70
April	67	60	112
May	84	63	133
June	63	55	115
July	91	79	115
August	63	81	78
September	86	76	113
Summer	454	414	110
Year	797	904	88

fact "perhaps the most abnormal period for the past thousand years" in terms of the British climate. What is important is that the CWPU, at least, takes the possibility of climatic change seriously. A spokesman said that they "would be very interested in seeing more work [of this kind] done, and are open to suggestions for programmes of research". The old Board has provided funds for research into the variability of rainfall in the English lowlands at the Climatic Research Unit of the University of East Anglia, but the present situation with regard to the continued funding of such work is not yet clear.

Under the situation which prevailed until April this year, the Water Re-

independence in water supply, and dotting the local countryside with reservoirs rather than 'importing' water from neighbours.

Indeed, the most nationalistic of the Welsh might press the case that England should be made to pay for 'Welsh' water—although the CWPU has a stock answer to that: build a dam across the border and see what happens if the Welsh do try to keep all their water!

In fact, of course, water from Wales is so important because of a coincidence of geography and climate. The water brought in from the Atlantic is dumped conveniently on the Welsh mountains, providing the possibility of storing it near the source of rivers and using it to regulate their flow. This makes schemes

to develop that potential still further more attractive than alternatives, such as barrages at the Wash or in Morecombe bay; indeed, one plan now being studied would involve expansion of the Craig Goch reservoir to produce 500 million gallons a day—more than twice the capacity of the Wash scheme in its latest form.

The Craig Goch scheme is representative of the change in philosophy of the water planners from the days when direct supply from reservoirs to consumers was fashionable. Now, the aim is to use rivers wherever possible, meeting the demand for abstraction by regulating the flow using reservoirs at the head of the river. This also improves the health of the river, as well as making it possible to take out more water than from a comparable 'wild' river.

Because of this philosophy of river regulation, the planners must now take increasing account of weather forecasting on a day-to-day basis. If water is released from a reservoir during a dry spell to maintain the flow of a river, it could be embarrassing if heavy rainfall a couple of days later were to occur lower down the river just as the surge from the reservoir arrived. This has provided one of the closest direct links between the old Board and the Meteorological Office in recent years, when both have collaborated with Plessey Radar Ltd and the Dee and Clwyd River Authority on a radar weather forecasting project which has formed part of the River Dee Regulation Research Programme.

Apart from the new outlook on surface water management—which depends very much on the cooperation of the new regional authorities—there are also plans to develop groundwater abstraction. In the tenth (and last) Annual Report of the Water Resources Board (HMSO, 79p) it is suggested that resources will be available to maintain the proportion of water derived from aquifer storage until the end of this century, even though total demand is expected to double by then. In the long term, there is the possibility of artificial recharge of aquifers from wells or by



Craig Goch reservoir

"infiltration" through lagoons or basins; pilot projects carried out in the London Basin, in Nottinghamshire, Sussex and other parts of England and Wales have shown the feasibility of refilling some strata with water and abstracting the water when required, and research into and development of such techniques must clearly have very high priority in the plans of the Water Research Centre and the CWPU.

But one superficially attractive proposal for the future seems to have been ruled out for some time to come. Desalination has been widely mooted as a practical system of obtaining pure water in Britain, because of the extensive coastline. But studies suggest that it will be simply too expensive in the fore-

seeable future (the next 25 years), especially when the rising costs of fuel are taken into account. In addition, the environment lobby, which by and large seems to favour the idea of desalination, has not, perhaps, taken full account of the impact on the coastal environment of unsightly desalination plants. Just about the only economically viable use of these plants in Britain at present would be to top up the supply of coastal resorts in the summer, when their population rockets and demand is highest (indeed, desalination is used in just this way in the Channel Islands). But on the other hand, the authorities of areas which depend on the income obtained from visitors attracted by the natural beauties of the coast are the least likely to authorise construction of new industry.

However the investigation of climatic change develops, the question of water supply is bound to occupy an increasingly important place in the thoughts of planners around the world as population increases. Desalination will obviously be important in some areas, if not in Britain. But the problems with desalination as a commercial proposition highlight the truth that although "two thirds of our planet is covered by water" we must think very carefully indeed about where we are going to get a drop to drink in the year 2001 □

Table 2 Water Resources Board expenditure on research

Financial Year	Research £	Section 90* £	Total £
1966-67	31,265	17,948	49,213
1967-68	41,260	41,553	82,813
1968-69	63,866	121,604	185,470
1969-70	120,878	107,998	228,876
1970-71	259,967	167,254	427,221
1971-72	226,679	194,027	420,706
1972-73	237,111	293,686	530,797
1973-74 (estimated)	436,300	347,700	784,000

* "Section 90" is the section of the 1963 Act which empowered the Water Resources Board to provide financial support for work and research carried out by the then river authorities.

international news

AN appeal to the scientific community by a committee of the National Academy of Sciences to refrain from conducting two types of genetic manipulation experiments because of potential hazards to society (see *Nature*, July 19) has drawn a swift and positive response from the National Institutes of Health (NIH). In a letter sent last week to Academy President Philip Handler, NIH Director Robert S. Stone indicated that he will establish a committee to define the possible hazards associated with such research and that NIH is willing to support an international meeting of scientists to discuss the matter.

The academy committee has urged that a moratorium should be placed on experiments which (I) involve the introduction into a bacterium of genes which either confer resistance to antibiotics or cause the formation of bacterial toxins and (II) those which involve the introduction of genes from viruses into bacteria. Furthermore, the committee suggested that experiments which introduce genes from animals into bacteria "should not be undertaken lightly".

The reason for concern is that the bacterium most commonly used for such studies is *Escherichia coli*, which

NIH backing for NAS ban

by Colin Norman, Washington

usually present in the human intestine. But the committee is quick to point out that the concern is "based on judgments of potential rather than demonstrated risk", and that the danger is yet to be precisely defined.

Each member of the committee, which was chaired by Dr Paul Berg of Stanford University, has agreed to eschew experiments of both sorts. And they have called on their colleagues throughout the world to join them in deferring such research until the hazards have been evaluated.

When the committee's statement was made public last week, Berg said that an international meeting is being planned for next February to "discuss whether there are in fact experiments that should or should not be done". The meeting, which will probably be attended by about 100-150 scientists, will not only attempt to define what types of research should be included in the embargo but also for how long it should be maintained.

Asked whether he expects that a

voluntary moratorium will be effective, Dr David Baltimore, a member of the committee, said that at present he knows of no laboratory which is planning to undertake experiments of type I or II, and that peer pressure on scientists now to eschew such studies will probably be sufficient to make the embargo stick. He pointed out, for example, that study groups at NIH—committees of scientists which provide initial peer review of grant applications—will be wary of approving funds for such studies and that the editorial boards of scientific journals will probably think twice about publishing research papers derived from experiments covered by the embargo. "To me", he said, "it is almost unthinkable that scientists would go out and do this type of work now".

The committee acknowledges, however, that its recommendations "will entail postponement or possible abandonment of certain types of scientifically worthwhile experiments". It therefore remains to be seen how long the scientific community will go along with this move toward self regulation, and much will depend on the outcome of the NIH committee's deliberations and next February's international meeting.

What British scientists say . . .

Molecular dirty tricks ban

THE critics of molecular biology are fond of pointing out the scarcity of practical benefits. It is a sad paradox that the very developments which could ultimately have immense value for production of useful, but rare, molecules, should result in an urgent cry of 'halt' from a committee of leading scientists involved, supported by no less than the United States National Academy of Sciences. The amplification of selected genes and their products by synthetic recombination with freely replicating DNA of bacterial plasmids, could, for example, revolutionise the commercial production of substances like insulin, or pituitary hormones, while the bulk synthesis of a transforming gene protein from an oncogenic virus might allow the design of specific antagonists. As more genes are identified on cleavage products of DNA from animal

cells and viruses, so the potential for practical application will increase, to say nothing of the basic knowledge gained.

Yet few will deny the wisdom of the appeal for a moratorium on certain classes of experiment until the implications are more clearly understood. It is not just the risk of unpredicted and explosive self replication of some sinister DNA sequences which causes concern, it is the possibility that by using *Escherichia coli*, the workhorse of molecular biology, the explosion might occur in someone's gut and then be transmitted like antibiotic resistance, throughout the world. No doubt a good many dirty tricks have been attempted and discarded by nature in the course of evolution, but the disquiet arises from the utterly novel associations of genetic material which are now possible. The potential benefits should, therefore,

be delayed, not for ever, but until consequences can be assessed, and preliminary experiments carried out under conditions of maximum security.

Most of the technology involved in all this has been developed in the United States and it is encouraging that the very leaders in the field have taken the initiative and have been supported by the academy. It is now to be hoped that academics and learned societies in other countries will add their weight, and that international organisations such as the European Molecular Biology Organisation will lend support. Granting agencies, and even editors of scientific journals, will also need to consider their policies in the face of wide support for a moratorium. For many it will be a test of self denial and social responsibility in the face of strong intellectual temptation.

Michael Stoker, ICRF

The indiscriminate use of antibiotics has exerted more pressure on the bacterial population than could be wielded by all research workers in the field put together.

from E. S. Anderson, Colindale

THE NAS statement on plasmid engineering evokes a mixed reaction from me. Whatever its justifiability, I wish it had been presented less pompously. We have been more aware since our first experiments in transferable resistance in 1964 that by introducing an R factor into a pathogen we reinforced this organism's pathogenicity with resistance to drugs that might be effective against the respective disease. In particular, when chloramphenicol resistance was introduced into the typhoid bacillus, we were manufacturing a virulent pathogen resistant to the drug of choice for typhoid fever. And we knew that if we presented such a strain with the opportunity of causing infection we would have been guilty of a grave antisocial offence. But R factors had become important in the enterobacteria and they demanded study. We could not do such work effectively if we shirked the step of introducing them into pathogens. So we transferred them to pathogenic enterobacteria, including *Salmonella typhi*, exercising our routine precautions for avoiding infection with the respective organisms, and for their safe storage. We have studied the typhoid bacillus and related organisms for many years, but no infections have resulted from our work, inside or outside the laboratory; so our protective measures must be reasonably efficient. Moreover, it is fair to say that these manipulations have contributed to the understanding of R factors and other plasmids, including the evolution of an easy method for establishing the plasmid nature of elements concerned with enterobacterial drug resistance, virulence or other properties.

The *in vitro* hybridisation of plasmid DNA with DNAs of widely different, even eukaryotic, origin, adds a new dimension to plasmid studies. Once introduced into the bacterial cell, the hybrid plasmids resume the relationships of their bacterial moiety and multiply with the cell. The eukaryotic regions, however, retain their original coding capacity and initiate the synthesis of the corresponding products.

What is the point of such experiments? First, they demonstrate that DNA-DNA hybridisation need not require extensive regions of base-pair homology. They therefore expose processes that may have important evolutionary significance or medical potential. Second, they may help to clarify the *modus operandi* and the nature of the products of the "Xeno-DNA", if one may coin such a term for the introduced foreign DNA. Normally, such

studies are hampered by the relatively slow cell growth and much greater complexity of the respective animals or

tissue cultures. In hybrid bacterial-animal plasmids, synthesis and replication are geared to the much higher kinetics of bacterial growth, so that the respective DNA and its products can be isolated in adequate quantities in a short time. This might accelerate the studies of these materials and could add

Alternative experiments?

SECTIONS of genetic material from any source can now be attached to a bacterial plasmid or bacteriophage and so introduced into the bacterial cell where they replicate quickly and can be recovered in relatively large amounts. By-passing the normal biological barriers between species in this way means that completely novel genetic combinations can be created and disseminated.

But it is at present impossible to predict all the properties and ecological consequences of these new genetic arrangements. Not unnaturally this has led to widespread concern over possible biohazards from such research, which has resulted in the National Academy of Sciences appeal. Concern is enhanced because the ubiquitous human enteric bacterium *Escherichia coli*, has been used as the organism in experiments carried out so far.

Anxieties arise on two main counts. (1) It is generally impossible to transpose selectively only those DNA fragments with known functions. Potentially harmful DNA fragments, such as those containing possible oncogenes, may unwittingly be transferred to a new host where their expression may not be properly controlled. (2) The vehicles used (in the work published so far) to transfer foreign DNA fragments to *E. coli* were plasmids that carry genes determining resistance to antibiotics.

The NAS embargo covers two types of experiment (which have already been accomplished by some of the signatories of the statement), namely the transfer of genes determining drug resistance between bacterial strains or species that do not normally carry such resistance, and the fusion of animal virus genes to transmissible systems.

The NAS request is both reasonable and responsible and deserves to be universally respected. It recognises both the difficulty in evaluating real or potential hazards that may be involved in such work, as well as the obvious criticism that these will remain obscure in the

absence of experimental study; urgent consideration of the latter is explicitly recommended.

Fears that the proposed limitations to experiments will seriously obstruct research in vital areas of biology seem unfounded. The NAS initiative, by focussing attention on the hazards involved, could well promote rather than hinder work on *in vitro* recombination in animal viral systems, an area believed by many to hold the key to gene therapy in its broadest terms. Similarly, the constraints on experiments with plasmids determining drug resistance need not preclude cautious use of the plasmids for cloning certain DNA fragments. Many experiments in this latter area can also be undertaken with bacteriophage λ which is a safer vector for *in vitro* recombination in that it has strict host specificity and carries no drug resistance determinants; it has the great additional attribute of bringing a wealth of genetic and biochemical experience to the service of studies on the cloned fragment of DNA. Use of phage λ does not, of course, eliminate hazards associated with the incorporation of unknown genes.

In view of the anxieties expressed, the wisdom of using a normal human enteric bacterium as host organism in these experiments might be questioned. Certainly there is no reason to believe that one could not select quite different microbial and viral systems that do not inhabit man or mammals, but the hazards, real or potential, would apply equally well to other ecosystems and thus eventually raise the same problem. To do this, however, would be to forsake the wealth of information and experience with *E. coli* and its viruses accumulated from decades of research. This would be prodigal to say the least.

While welcoming the NAS initiative, one is also appreciative of Anthony Tucker's perspective in his comment "Life Stylists" (*The Guardian*, July 19); equally, if we follow the moderate tone set by the NAS we shall be careful not to oversell the social benefits devolving from recent experiments.

Ken Murray, Edinburgh

substantially to the understanding of the respective systems. By hybridisation of oncogenic viral genomes with bacterial viruses or plasmids, cancer workers could perhaps selectively enrich the return of oncogenic material for study.

The signatories of the NAS letter define two types of experiment on which they recommend a moratorium. Type I comprises experiments in which plasmids coding for drug resistance or potential pathogenicity are introduced into pathogens or innocuous bacteria. The fear is of the risk to treatment if a newly resistant pathogen reached its host; or of the emergence of new pathogens that might become ecologically important. *In vitro* construction of plasmids for new drug resistance would also be suspended.

Type II experiments in the NAS letter are those involving hybridisation of plasmid with eukaryotic, or oncogenic or other viral DNA. Such hybridisation might create hazards of an unforeseen nature, since the potential of the hybrid genome cannot be accurately predicted. Hybrid plasmids may thus have oncogenic or other potential for man or other animals. And since the enterobacteria, most widely used for plasmid study, are ordinary inhabitants of the human or animal intestine, they may by accident or design enter the intestine of a suitable host, spread, and act as the vectors of cancer and related diseases, perhaps with augmented virulence or on an epidemic scale.

A sense of proportion must be retained in considering these matters. The widespread and indiscriminate use of

antibacterial drugs in man and animals has exerted immeasurably more pressure on the bacterial population than could be wielded by all the research workers in this field put together. These drugs are used prodigiously in human and animal medicine, and as animal feed additives. The long-predicted results, now realised, were the spread on a gigantic scale of transferable drug resistance plasmids in the ordinary intestinal bacteria such as *Escherichia coli*; and the emergence of epidemic drug-resistant and lethal lines of enterobacterial pathogens: *Shigella dysenteriae* 1 (Shiga's bacillus) in Central America, the typhoid bacillus in Mexico, India, South Vietnam and Thailand, and *S. typhimurium* and other salmonellae with massive drug resistance and apparently increased virulence in many countries. This has been the direct consequence, not of plasmid work, but of commercial pressures and administrative and professional irresponsibility.

For these reasons I feel little inclination to respond to the call for a moratorium on type I experiments in the NAS letter.

Hybrid plasmids formed by the introduction of normal, and oncogenic and other viral DNA, may present a potential threat, but it is difficult to see how this could be evaluated. Experiments on animals what are the natural hosts of particular viruses spring to mind. But these may not reveal potentialities of the hybrids that might be expressed in different animals. Naturally, tissue culture would be used and the range of experimental animals would include primates, but if negative results are obtained in these, how and

when can we be sure that the chimaeric plasmids are innocuous to man or other animals? The risks cannot be quantified as can energy release from nuclear reactions, because the processes are biological and complex, not physical and simple. And the techniques needed for reliable exposure of oncogenic potential may not yet have been devised.

There is therefore a need for caution in the type II experiments. Bacterial vectors of hybrid plasmids can, however, be adequately controlled by the ordinary sterile techniques of laboratories working with pathogens. This would call for technical re-education of the average microbial geneticist or molecular biologist, whose manipulation of bacteria chills the blood of anyone accustomed to handling pathogens. Cultures should be stored securely: methods for doing this already exist. Such precautions, which should be routine, minimise the risk of accidental spread of dangerous pathogens, and no technical distinction should be made between known pathogens and presumed non-pathogens.

Strict isolation techniques must be practised if animal experiments with bacteria carrying plasmids in the type II category are carried out.

The methods needed to maximise safety in such investigations are known, and plasmid researchers must learn them if work on these hybrid plasmids is to proceed. The only alternative is the abandonment of type II experiments, or at least a chosen proportion of them, which may mean the closure of potentially valuable avenues of research. There is a manifest need for expert international debate in this field.

THERE is a particularly irritating television commercial in the United States which contains the classic line that "all aspirin is not alike". Although that phrase was coined on Madison Avenue to make money rather than to make a scientific point, it is typical of the claims that are flying around in a bitter dispute between pharmaceutical manufacturers and the federal government over the quality and price of American drugs.

The dispute is simple enough. The government, backed by consumer groups, argues that a particular drug should produce the same effects no matter who manufactures it, and so government agencies should not throw money away by buying an expensive brand-name drug when the same product is available at a lower price.

But many drug manufacturers take a different view. They maintain that products containing the same ingredients but manufactured in different ways may produce different therapeutic effects. Therefore, they argue, if the

Differences between the same drugs

by Colin Norman, Washington

government insists on buying the lowest priced drugs it may also be getting the lowest quality products.

Last week, the Office of Technology Assessment (OTA), Congress's new scientific think tank, stepped into the debate. In its first report, prepared by a panel of ten eminent biomedical scientists, OTA provided at least partial backing for the arguments of the drug industry by stating that many drug products are not interchangeable, chiefly because shoddy manufacture and poor enforcement of quality standards by the federal government do not guarantee therapeutic equivalence.

The OTA panel, chaired by Dr Robert W. Berliner, Dean of the School of Medicine at Yale University and former Deputy Director of the National Institutes of Health, cited a number of

"well-documented and significant differences" between drug products that are supposed to be chemically equivalent. The differences, in fact, were not confined to drugs made by different manufacturers, but also cropped up among different batches of drugs made by the same company.

Perhaps the most clear-cut and alarming incidence of such disparities came to light during a study carried out at a New York City Hospital in 1971, which was singled out in the OTA report. That study turned up evidence that four different brands of a highly potent heart drug, digoxin, were so strikingly different in their effects that the peak concentration of the drug in the bloodstream differed by a factor of seven. Yet the margin of safety of digoxin is so narrow that lethal effects can occur if the dose given is only twice that needed to produce a therapeutic effect.

The OTA panel reckons that there is probably "roughly a score of drugs" for which there is evidence of dif-

ferences in so-called bioavailability among brands which are supposedly chemically equivalent. And it suggests that such differences have resulted in therapeutic failures or inadvertent poisonings when patients have been given doses containing too little or too much of the prescribed drug.

The importance of the OTA's findings is that they have a direct bearing on proposed new policies governing payment for drugs by the federal government. On December 19 last year, the Secretary of Health, Education and Welfare, Caspar Weinberger, sent a shiver of apprehension through the pharmaceutical industry by announcing that the federal government would limit the price paid for drugs under the state-run Medicaid and Medicare programmes to "the lowest cost at which the drug is generally available". About \$1,300 million is paid each year for prescription drugs under those programmes, and Weinberger estimated that the new policy would save between 5 and 8% of the costs.

But last week the Pharmaceutical Manufacturers' Association (PMA), the trade association which represents nearly all the big United States drug makers, claimed that the OTA report "completely undercuts the ill-advised proposal" for drug purchasing, because it challenges the basic principle that chemically equivalent drugs are interchangeable.

Berliner pointed out last week, however, that for most drug products biological equivalence is not critical because there is a wide margin of safety between the dose needed for therapeutic effects and the toxic dose. He reckoned that only "about 10 to 15%" of the drugs on the market may pose a problem because of therapeutic differences between brands.

Science studies in Amsterdam

by a Correspondent

At a time when the values of scientific activity are increasingly being questioned, so social studies of science are being included as optional or compulsory courses in universities round the world. British experience in this field—particularly that at the Universities of Edinburgh, Manchester and Sussex—has achieved considerable note during the past few years. So therefore it comes as no great surprise, even in these times of fluctuating interest in European collaboration, that the programme of teaching and research in science studies at Edinburgh will be the model for an unusual new unit at the Free University in Amsterdam. Moreover, British and Dutch collabo-

Confiscated manuscripts

by Vera Rich

THE scheduled date of the Moscow "seminar that never was" has passed, and the would-be organisers and participants have been, at least temporarily, released from custody—yet the history of this valiant venture in academic freedom seems far from complete. As might be expected, the attention of the authorities is still focused on Professor Aleksandr Voronel, whose Moscow apartment was to have been the venue of the seminar. On Friday July 19, 1974, his apartment was subjected to an 8-hour search by the

police who confiscated some 2,000 papers, mostly scientific manuscripts, including those submitted by intending participants in the international seminar. On the same day, the fourteen-year-old son of Dr Benor Gurfel, another of the seminar sponsors, was stopped in the street on his way to deliver certain manuscripts to Professor Voronel. These were also confiscated.

Professor Voronel and his wife are under constant and obvious surveillance, and are at present staying away from the apartment. This confiscation of manuscripts is seen by informed observers as an ominous sign—the intensive campaigns against major dissidents such as Solzhenitsyn began in precisely this way.

ration is further strengthened by the appointment from August of a British historian of science and former palaeontologist, Dr Martin J. S. Rudwick, from the Department of History and Philosophy of Science, Cambridge, as the unit's first director and also as the first professor of History and Social Studies of Science in the Netherlands.

The Free University has long had a liberal attitude to the education of its science students. A course in general philosophy has been compulsory since the university's foundation in 1880 and history of science was added to the curriculum of all students in the late 1940s on the appointment of the noted historian of science, Dr R. Hooykaas, as professor. When Professor Hooykaas retired—about the time of the student unrest in the Netherlands at the end of the 1960s—it was decided that it would be expedient to widen still further the scope of the curriculum by introducing courses in science and society. For the past two years staff from the History and Social Studies of Science Division at the Science Policy Research Unit, convened by Dr R. M. MacLeod, have helped Dr E. Boeker, Professor of Physics in the Natural Sciences Laboratory, to prepare the ground for the new unit which will be the cornerstone of the Centrum Algemene Vorming (Centre for General Education) in the Faculty of Mathematics and Natural Sciences.

All first and second year students in the five departments of the faculty must now attend 52 lectures a year on philosophy, and the history of 'science and society'. Since 1972/73, teaching has been divided between the staff of the science and philosophy departments and visiting scientists from industry as well as guest lecturers from Sussex. The Sussex staff have also assisted with the seminars for the science and society 'minor' option

which graduate students can take during their fourth and fifth years.

When Dr Rudwick assumes his appointment in August, the unit will then start acting as general co-ordinator of all the courses in general studies, including those at present taught by the different departments—the so called encyclopaedia. The first PhD student in science studies at the Free University (a physicist who is currently taking the MSc course at Sussex) will register next year and it is hoped that in time the unit will be able to carry out a regular programme of research. Dr Rudwick's present interest is the social and conceptual history of science; to complement this, it is expected that staff who specialise in the sociology of contemporary science will eventually join him in the Centrum.

Although science studies and science policy units are found in other universities on the continent—for example, at Heidelberg and Lund—they are usually separate from the central core of the science faculty. At the other five universities in the Netherlands, some science and society courses are available, but these are not as widely based as that offered at the Free University in Amsterdam. Chemistry students seem to be the most privileged in this respect; at the State University of Groningen, for example, the chemistry department offers a major in 'free chemistry' with courses in chemistry, physics, economics and sociology. There has been some success in introducing discussions on ethical problems into biology courses, but in physics and mathematics departments there has been more resistance, though in the department of physics at the Technical University, Eindhoven, students can do a minor in physics and society.

No takers

A WEALTHY businessman's plans to give grants to young scientists for basic biological research ended in disenchantment on both sides at a meeting in London recently, when it transpired that he would not consider any applicant who held any sort of religious belief.

Mr Ray Turner had hoped to set up a foundation to award grants to young biologists under 28 years old for "independent fundamental research into outstanding questions relating to the chemical organisation of life". The grants were to be at a rate 20% above the United Kingdom universities non-clinical academic scale with tenure for 5 years initially and the opportunity for repeated extension; in effect allowing the possibility of a lifetime's financial support.

Thirty-six applicants who, under the terms of the application, had been nominated by senior researchers, assembled at the Strand Palace Hotel at Mr Turner's expense for an informal meeting to present their research proposals for selection.

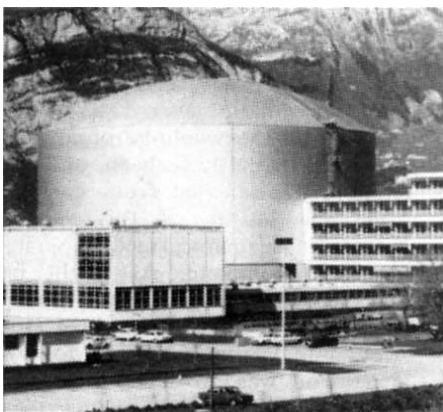
As most of them expected some sort of scientific panel, they were surprised to find only Mr Turner, his wife and Professor Bellamy of the Zoology Department at University College Cardiff, the 'scientific coordinator' who had signed the original circulars inviting applications which were sent to university heads of departments in early June.

Each applicant was asked for a brief resumé of his work and aims, and his ideas of "the single basic facet of the really great scientist". Mr Turner then said that he wished to ask a question which might surprise some people but that the motive would be explained later.

He then asked if anybody had religious beliefs. Four applicants put up their hands and their names were noted. The rest were then asked whether any of them held any belief in a "supernatural plan". More put up their hands and their names were also noted. The remainder resolved, after lively discussion, into what Turner classed as agnostics and atheists.

As it had become clear by now that selection was not going to be made on conventional scientific grounds, most of the participants thought that this might be some sort of 'management selection technique' but during lunch Mr Turner had a private discussion with one of the participants, Peter Leadley, who had put up his hand in the first group. Mr Turner told him that although he was considered among the most promising of those present, he could not be considered for a grant on account of his religious beliefs.

Now it's legal



Reactor in the Alps

It was a symbolic occasion at Grenoble last week as the ministers from Great Britain, France and West Germany signed an agreement bringing Britain into equal partnership with the other two countries in supporting the high flux neutron beam facility at the Institut von Laue-Langevin. Symbolic, because the partnership has existed *de facto* since January 1973. And yet, such is European collaboration on other things that since then the three countries have been trying hard to find a convenient date for ministers to participate in a ceremony giving tangible evidence that in some fields an international spirit still prevails.

The institute is hardly unfamiliar with symbolism; it exists in large part because France and Germany needed some collaborative scheme in the mid-1960's to patch up relations—indeed folklore goes so far as to say that Professor H. Maier-Leibnitz was asked to propose a project, suggested a high flux reactor and so it was. Not that it is in any way a white elephant. There was an urgent need for such a facility to further research in all fields from nuclear physics to biology, and it is

widely agreed that the institute is fulfilling its purpose admirably.

The 57-MW reactor was built by French and German firms and first went critical in August 1971. It delivers a maximum flux of 1.5×10^{15} neutrons $\text{cm}^{-2} \text{s}^{-1}$ and, in addition to supplying thermal neutrons to up to 50 separate experiments, has a cold source—25 litres of liquid deuterium as a low temperature (25 K) moderator—and a hot source—10 litres of graphite as a high temperature (2,000 K) moderator.

The main use of the neutrons so far has been in the study of condensed matter. Neutron diffraction can determine crystal structures, including the location of light atoms which are inaccessible to X-ray diffraction. Long wavelength neutrons are used to study defects and biological structures. The magnetic moment of the neutron is a valuable tool in understanding magnetic materials, and inelastic scattering is being used in studies of the dynamics of atoms and molecules.

As would be expected the scientific staff is international in character, although the British, who have been pioneers in neutron techniques, face problems in uprooting themselves for a period of several years in that return to a Britain with a depressed job market in high-flying science is bound to cause worries. On the other hand British technicians there speak with enthusiasm of their conditions and salary and some are prepared to stay indefinitely.

The running costs of the reactor and institute are £6 million this year but Professor Mössbauer, the director, is seriously concerned about inflation. Commitments in the newly signed document are inadequate for inflationary times and it has not been possible to hammer out tripartite agreement on including inflation automatically into each country's contribution. "As soon as the ink is dry", he said "I have to go back to the sponsors to negotiate more money for next year".

While the scientists were still debating whether even this might be some sort of psychological selection test, Mr Turner announced that he could not consider any of the applicants who had expressed any belief in a religious or supernatural power.

Mr Turner first said that he did not wish to give his reasons but during the three and a half hours discussion that ensued he eventually explained that he felt that if a scientist believed in any sort of supernatural power he would experience conflict in researching problems which enquired into the fundamental nature of life. He therefore thought that the very best scientists, the ones he wished to encourage, could not be religious.

At this point, Professor Bellamy who had taken little part in the proceedings so far, stated his opposition to the method of selection as did several of the 36 scientists, some of whom then left. The rest, according to a participant who stayed, tried to make Mr Turner realise that his sort of criteria could not be used as a basis for selecting scientific work and that it would be unacceptable to the scientific community at large. Mr Turner was asked whether he would consider handing over the money to scientific trustees to administer, but he refused.

Eventually it became obvious that deadlock had been reached and Mr Turner announced that he was withdrawing his offer.

news and views

Nutrition and immunity

As is demonstrated by the communication of Aschkenasy on page 325 of this issue of *Nature*, there is currently a broad focus of interest in the relations between nutrition and immunology. At first glance the association of these two disciplines may seem strange; their union grew from clinical and epidemiological experience in malnutrition and infectious diseases, experience that eventually led to the understanding that either of these conditions tended to augment the other. Many observations, such as that of an increased morbidity and mortality from measles among sub-optimally nourished children, suggested that nutritional deprivation tends to render persons more susceptible to certain disease, and suggested that some aspects of host resistance are functionally impaired. Meanwhile, laboratory workers were amassing evidence in support of the concept that nutritional manipulations could indeed affect the immune response. Much of the clinical and laboratory work concerning the nutrition-infection-immunity cycle was published in 1968 in a classic World Health Organisation monograph (*Monograph Ser. W.H.O.*, No. 57). As it became generally acceptable that the immune response accounts for much of host resistance, it also became more reasonable to ask if malnutrition affects the immune response. Answers to this type of question required the collaboration of nutritionists and immunologists, and forged a new direction for both sciences.

Studies of nutritional-immunological relationships in man are difficult, for many reasons. In the first instance, pragmatists may assert, with some justification, that the answer to malnutrition is to provide more food. This is of course true, but until more food is provided the problem will exist, and famine and pestilence will continue. Another approach to the problem might be gained by asking what particular components of host resistance are affected by malnutrition, and by the subsequent use of modern immunology, nutrition or public health either to repair the lesion specifically or to circumvent the need for its function. Some persons have maintained that the problem is too complex to study because of the great number of uncontrollable variables. Logistics have been another impediment to the study of nutritional-immunological interactions in man. Malnutrition often exists in areas where medical and research facilities are limited, where weather and terrain are difficult and where many other social and medical problems require priority. In spite of these difficulties, several national and international centres and institutions have begun to construct a matrix of collaboration and communication with the purpose of defining and dissecting some of the effects of malnutrition on the immune response.

For the most part, these studies have sprung from individual efforts, but many of the investigators have elected to coordinate aspects of their studies through the World Health Organisation (*Bull. Wld Hlth Org.*, **46**, 537; 1972). The WHO has responded in its usual efficient and effective way by formulating an international collaborative study (*Amer. J. clin. Nutr.*, **27**, 638; 1974), the fabric of which has served to strengthen collaboration between national and international bodies, and to extend communications be-

tween field and laboratory workers. More information about this can be obtained by writing directly to the World Health Organisation in Geneva.

Although a great deal of work remains to be done, data from field and laboratory studies thus far do seem to be piecing together a preliminary but coherent picture of the immune response in malnutrition. For example, amounts of serum immunoglobulin (Ig) do not seem to be affected consistently by protein-calorie malnutrition (PCM), but elevated levels, especially of IgA, are not unusual (*J. Pediat.*, **81**, 1194; 1972). Increased values for Ig in PCM have been attributed to intercurrent infections (*Archs. Dis. Childh.*, **45**, 282; 1970). This seemingly increased synthesis of Ig needs to be reckoned with data that convincingly show serum albumin (*Lancet*, **i**, 63; 1973) and most components of the blood complement system to be depressed in PCM (*ibid.*, **i**, 1016; 1973). Amounts of antibody in malnourished persons are also inconstantly affected, but in these circumstances the effect seems to be antigen-dependent; for instance, vaccination against polio or measles produces measurable antibody (*Archos lat-am. Nut.*, **23**, 345; 1973); but some viral and several bacterial antigens do not produce a high incidence of seroconversion (*Trop. geogr. Med.*, **18**, 125; 1966). These observations would seem to be relevant to current vaccination programmes. They also suggest a clear need for more applied and basic research into problems such as the effectiveness of dietary supplementation before vaccination, the route of immunisation, the type of adjuvants, and the relative T- or B-cell dependance of antigen for human use.

Studies of cell-mediated immunity (CMI) in human malnutrition have suggested that the thymus-derived components of the immunological system are damaged. Indeed, histopathological studies have reported a virtual absence of thymus tissue (*Envir. Child Hlth*, 217; September 1972). These morphological alterations seem to be mirrored in tests of T-cell function; for instance, results of skin testing for delayed hypersensitivity reactions to many antigens, particularly to tuberculin following BGG vaccination (*Lancet*, **ii**, 719; 1965), have been reported to be significantly depressed (*ibid.*, **i**, 506; 1973). Insofar as positive tuberculin reactions are thought to be associated with protection following BCG, the absence of such reactions is likely to be relevant to the incidence of tuberculosis among sub-optimally nourished persons. Other measures of CMI have also been reported to be depressed in malnutrition. The response of human peripheral blood lymphocytes to phytohaemagglutinin *in vitro* is generally regarded as a measure of T-cell function, and this reaction is reportedly suppressed in PCM (*Lancet*, **ii**, 939; 1971). Numbers of T cells can also be measured *in vitro* by allowing them to form rosettes with other cells, and results using this test have again suggested that malnourished persons have diminished numbers of T cells. The message of these several lines of evidence seems to point to a type of T-cell immunodeficiency in malnutrition. If this is true, it does not, of course, alter the dictum that the answer to the cycle of malnutrition and infection is to provide more food, but it does suggest that perhaps the cycle can be prophylactically or therapeutically approached from another bias.

Several biochemical and cellular events important in the nutrition-immunity-infection cycle have also been studied. Biochemical studies of phagocytic cells from malnourished

persons have reported a depressed glycolytic pathway (*Amer. J. clin. Nutr.*, **24**, 272; 1971), and these defects are reversed when patients are given a diet rich in calories and proteins (*Biochem. J.* **127**, 255; 1972). Functional studies of similar cells have reported a delay in the killing of phagocytosed bacteria, and this defect is also reversed by adequate diet (*Clin. Exp. Immunol.*, **17**, 121; 1974). Another defect in the killing of phagocytosed bacteria is corrected simply by the addition of iron (*Archs. Dis. Childh.*, **48**, 863; 1973). If these various defects represent different mechanisms which are corrected by different types of dietary supplementation, it would seem to be wise to know more about the mechanisms involved. The bacterial-killing defect, which is presumably lysosomal, may be related to the immunodeficiency discussed earlier; for instance, one mechanism for activating macrophages to kill phagocytosed bacteria is by way of products from antigen-activated T cells. This type of basic information is being integrated into modern nutrition and public health programmes, the practical effects of which are yet to be determined.

A great deal of work remains to be done. Almost nothing is known about the effects of intrauterine malnutrition on the development of the immunological system, the long-term effects of early malnutrition on the immune response, or the effectiveness of vaccination programmes in marginally nourished populations (*Lancet*, **ii**, 675; 1972). Answers to these questions would seem to be relevant to public health programmes in both developing and developed countries. Many of these questions can be addressed to animal models as has been done by Aschkenasy (*Immunology*, **24**, 617; 1973) and many others. This brief comment has not covered experimental models because of their obvious importance. Neither immunologists nor nutritionists pretend to have an answer to the malnutrition-infection cycle, but their combined approach has broadened understanding of an area of human biology that concerns us all.

W. PAGE FAULK

Evolution of nucleotide-binding proteins

ONE of the most fascinating questions about the formation of life and its immediate development is how the enzymes needed to sustain the basic life processes were derived. It seems reasonable that the relatively large number of enzymes required by even a primitive bacterium were derived from a much smaller number of basic, relatively non-specific enzymes—probably by gene duplication and subsequent divergence leading to a diversification of function and a narrowing of specificity. On this basis it would be expected that the large number of present-day enzymes could be grouped into a much smaller number of families, each of which was derived from a common ancestor. But if the common ancestor is very remote, there may be insufficient indication from the amino acid sequence to establish relatedness. On the other hand, since it is known from the evolutionary studies of the globins, the serine proteases and the cytochromes that tertiary structures change very slowly, X-ray diffraction studies of protein structure seem to be the most promising method for identifying family relationships. During the past year or so X-ray structure determination of a number of intracellular metabolic enzymes have produced some exciting results that seem to provide the basis for characterising an extensive family of nucleotide-binding enzymes.

The structural relationship was first seen clearly when the structures of liver alcohol dehydrogenase (LADH) (Brändén, *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2439;

1973) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Buehner *et al.*, *ibid.*, 3052) were compared with the homologous lactate and malate dehydrogenase (LDH and MDH) molecules. The polypeptide chains of all four dehydrogenases, composed of between 327 and 374 amino acid residues, are folded into two distinct units or domains. One of these domains is responsible for binding the common NAD cofactor and the other domain carries the binding site for the specific substrate of each enzyme and its catalytic site. In all four molecules the NAD-binding unit has a common fold that is characterised by a β -sheet core composed of six parallel strands (A-F), with a strand order CBADEF, and by a similar disposition of the mainly α -helical intrastrand loops above or below the sheet. The NAD cofactor is bound to the domain at an equivalent site in all four molecules. Earlier, Rao and Rossman (*J. molec. Biol.*, **76**, 241; 1973) had found that this domain seemed to be composed of two similar three-stranded units each associated with the binding of a mononucleotide, and they proposed that this unit was utilised to bind flavin mononucleotide in flavodoxin and to form the aromatic specificity site in subtilisin. In sharp distinction to the similarity of the NAD-binding domain, the catalytic domains, except in the case of LDH and MDH, are structurally quite distinct.

This pattern of relationship was subsequently observed in phosphoglycerate kinase (PGK) (Blake and Evans, *J. molec. Biol.*, **84**, 585; 1974; and Bryant, Watson and Wendell, *Nature*, **247**, 14; 1974), where the suggestion that the ATP binding domain of PGK has the characteristic fold and binding site of the NAD binding domain has been confirmed by the latest results. As with the dehydrogenases the catalytic domain has a distinct structure. The nucleotide-binding domain corresponds to the N-terminal part of the chain in LDH, MDH and GAPDH and the C-terminal part of the chain in LADH and PGK.

This pattern of structural relationships points strongly towards the evolution of the NAD-binding protein by gene duplication of the mononucleotide binding unit and its subsequent incorporation in the dehydrogenases, and probably in a modified form in the kinases, by a genetic fusion process involving protein fragments carrying the specific binding and catalytic properties of each particular enzyme. This hypothesis can only be established by amino acid sequence comparisons. Those comparisons of dehydrogenase sequences that have been made failed, however, to reveal this striking relationship, probably because the remoteness of the common ancestors makes it very difficult to align the sequences.

Rossman, Moras and Olsen in last week's issue of *Nature* (250, 194; 1974) reported that these problems could be overcome by using the structural and sequence data together to trace the evolutionary history of the nucleotide-binding unit. By superposing the hydrogen bond arrangement in the β -sheet core, they have been able to determine homologous amino acids in the sequences of the various dehydrogenases, flavodoxin and subtilisin. After aligning the sequences in this way, the calculation of the minimum base change per codon was used as a measure of the elapsed time since the occurrence of a common ancestor. The evolutionary tree produced in this way is consistent with the divergent evolution of the nucleotide-binding unit of the dehydrogenases and flavodoxin from a common ancestral binding protein. This common ancestor seems to be exceedingly remote and Rossman and his colleagues suggest that it is older than 3.2×10^9 yr, indicating that it may have been present during precellular evolution. Though evolutionary relationships need many data to establish them with any certainty, this analysis seems to indicate a line of protein development that is as old as life itself, which may link together two of the largest groups of enzymes, the dehydrogenases and kinases. At the same time it shows that

gene fusion may be an important process in molecular evolution, particularly as it gives the resultant molecule a binuclear structure that is an almost universal feature of enzyme molecules.

The structures of two other enzymes that may also be members of this family have been reported recently in *Nature*. In this week's edition, Campbell, Watson and Hodgson (*Nature*, **250**, 301; 1974) describe the structure of phosphoglycerate mutase (PGM) determined by a 3.5Å resolution electron density map. The molecule is a tetramer of four identical subunits, whose polypeptide chains are about 220 residues long. The core of each subunit is formed by a 6-stranded β sheet, in which four parallel strands are followed by an antiparallel pair, flanked by five of the six α helices in the subunit. Though the order of the β strands is not the same as that in the nucleotide-binding unit, the linear order and spatial arrangement of the five flanking helices is remarkably similar to that of LDH, although PGM has no known nucleotide-binding capacity. The authors show how the strands in the β sheet may have been rearranged if PGM has evolved from an ancestral protein with LDH folding, but at the same time point out that the general helix-sheet structure observed in these molecules may represent a particularly stable fold and imply, therefore, that it could be the property of more than one enzyme family.

The other enzyme, adenylate kinase (AK) was the subject of three papers in *Nature* for July 12. In the first, Schulz, Elzinga, Marx and Schirmer (*Nature*, **250**, 120; 1974) reported the structure of AK obtained from a 3Å resolution Fourier map. The enzyme's single polypeptide chain has 194 amino acid residues and it is folded into two domains, the larger of which has a five-stranded parallel β -sheet core with a BCADE strand order. Although X-ray analysis of substrate binding has not yet been carried out, the active site histidine is found near two hydrophobic pockets in a position equivalent to that of the adenine and nicotinamide binding sites in the NAD-binding unit, that may represent the AMP and ATP binding sites. In the second paper Schulz and Schirmer (*Nature*, **250**, 142; 1974) report the use of a method for comparing AK with other potentially related molecules that is based on the topologies of their

polypeptide chain folds. They define a "figure of topological relatedness" as the triple product of the number of ways of ordering the strands in a β sheet, the number of ways the connecting loops can be disposed on either side of the sheet and the number of possible locations for binding sites. The figure of relatedness is highest when AK is compared with subtilisin and lower, but still significant when AK is compared with flavodoxin or the dehydrogenase nucleotide-binding unit.

Schulz and Schirmer point out that topological comparisons cannot be used to distinguish convergent and divergent evolutionary relationships. But a more fundamental problem in using topologies or defining structural pathways to relate molecules with somewhat different chain folds is that such methods are not supported by any firm knowledge of the chain folding process. The best that can be done in this direction is demonstrated in the third article on AK, by Schulz and the "Who's Who" of the structure prediction world (*Nature*, **250**, 140; 1974). Using the joint probability of up to ten different prediction methods, they show that α helices, β -sheet strands and reverse turns can be predicted from the amino acid sequence with a fairly high degree of success, for this particular molecule at least. Although this is very promising, there is still a long way to go before, for example, the strand order in a β sheet can be predicted. Until this can be done structural comparisons must be treated with caution. Unlike its amino acid sequence, the tertiary structure of a protein reflects changes in the genetic material in an essentially unknown way and is likely to undergo discontinuous changes, such as the rearrangement of the order of strands in a β sheet, that have no intermediates. In these circumstances it would be unwise to use structural comparisons as more than indicators of possible evolutionary relationships. Though such relationships can only be established by sequence comparisons they are best carried out in the light of the structural information. It is by no means impossible that within this large group of apparently closely and more distantly related enzymes there are both homologous and analogous relationships, and it is vitally important to distinguish them if the path of molecular evolution is to be accurately traced.

C. C. F. BLAKE

State of play in the neural hypothesis of muscular dystrophy

THE most striking clinical and pathological change in the muscular dystrophies in man is the severe and progressive degeneration of the skeletal muscles in the absence of clinically or pathologically significant peripheral nerve abnormality. The motor nerve, however, is known to have a very marked effect on the function and metabolism of the skeletal muscle. The finding by McComas and Mrozek¹ that 27% of skeletal muscle fibres in mice with muscular dystrophy were denervated suggested that the motor nerve may have an even more important part to play in regeneration. Further studies showed a marked decrease in the number of motor units in the extensor digitorum brevis muscle in Duchenne and other human muscular dystrophies²⁻⁴, and in murine muscular dystrophy⁵. As a result of these studies, McComas and colleagues propounded the hypothesis that muscular dystrophy is caused by the "sick" motor neurone, the abnormal influence of which on the skeletal muscle fibre caused it to break down.

This hypothesis received support from the experiment of Salafsky⁶ in which muscle transplanted from dystrophic mice into normal hosts regenerated with the physiological characteristics of normal muscle. Further support came from

Gallup and Dubowitz⁷ who found that dystrophic mouse spinal cord would not support the regeneration of muscle in tissue culture, whereas normal spinal cord supported full regeneration both of dystrophic and normal muscle.

Much of this original evidence in support of the neural hypothesis has now been called into question. Harris and Marshall⁸ have failed to confirm the finding of denervated fibres in murine muscular dystrophy and have suggested that the original findings by McComas and Mrozek could have resulted from hypoxia of the preparations leading to artefactual presynaptic failure. Panayiotopoulos and colleagues^{9,10} and Ballantyne and Hansen^{11,12} have both criticised the technique used for motor unit recording by McComas and colleagues, and by more refined techniques have found that the number of motor units in the extensor digitorum brevis muscle of patients with various muscular dystrophies is normal. Several groups of workers have obtained results entirely contradictory to those reported by Gallup and Dubowitz^{13,14}. Preliminary results in my laboratory suggest that the contraction believed by Salafsky⁶ to arise from the transplant may in fact have been transmitted from the host's underlying normal muscles to which the transplant

is intimately fibrosed (unpublished work of A. Montgomery, W. G. Bradley and M. Jenkinson). Abolition of the contraction of these host muscles virtually abolishes the tension produced by the transplant.

Despite the contradiction of many of the basic observations leading to the neural hypothesis, experiments which bear on the nerve in muscular dystrophy are still being performed. In reviewing these it is important to remember that there are several different diseases both in man and in animals which have been classed under the title of the muscular dystrophies, and that the aetiology of each may be different. Desmedt and Borenstein¹⁵ found that motor nerves in patients with Duchenne muscular dystrophy are quite capable of sprouting to reinnervate denervated muscle fibres, and thus show no sign of being 'sick'. The total number and structure of the anterior horn cells in human Duchenne¹⁶ and murine muscular dystrophy¹⁷ are normal. Focal peripheral nerve abnormalities are found in murine muscular dystrophy but not in Duchenne dystrophy or in dystrophic hamsters¹⁸. The peripheral nerve abnormality in mice both of the 'severe' (dydy)¹⁸ and 'benign' (dy^{2j}dy^{2j}) (my and M. Jenkinson's unpublished work) forms of muscular dystrophy consists of a decrease in the number of Schwann cells and apparent arrest of myelination at the foetal stage of axons in the anterior and posterior roots, and the proximal parts of certain peripheral nerves. Axons run through these areas to become myelinated distally. My group has found that this abnormality of Schwann cell investment and myelination is present in the lumbo-sacral and cervical anterior and posterior roots, disappears with normal myelination in the region of the dorsal root ganglia, but recurs several millimetres distally in patches in the upper part of the lumbo-sacral plexus. We have also found it in the facial and trigeminal nerves.

These dystrophic mice also show an abnormality of axoplasmic flow in the peripheral nerves, with a decrease in the amount of slow flowing protein¹⁹ and of choline acetyl transferase²⁰, and an increase in the amounts of faster flowing proteins, phospholipids and cholesterol^{19,21,22}, with a probable decrease in the amounts of the fastest ('superfast') flowing proteins²¹. An abnormality of the peripheral nerve proteins probably derived from myelin has been reported in dystrophic mice²³.

Peterson's technique²⁴ of chimaera formation from normal and dystrophic mouse morulae is of considerable interest. He used isozymes to follow dystrophic and normal nuclei in the mouse which developed from these morulae. Some skeletal muscles had up to 94% of dystrophic nuclei, and many individual muscle fibres had only dystrophic nuclei. Despite this there was little or no pathological degeneration of these skeletal muscles. Conversely in some of

these mixed chimaeras, muscle containing no dystrophic nuclei sometimes showed pathological changes. The data suggest that in these mice the muscle degeneration is caused by some extramuscular abnormality. Peterson suggested that this might lie in the motor neurones, but it could equally be some abnormality conveyed in the blood stream.

This study is very important, and it must be extended to other enzyme markers, and to other animal models of muscular dystrophy. It is important to remember that the study does not necessarily apply to man and that the short history of the neural hypothesis is scattered with apparently conclusive studies which fail to be confirmed by other workers. Thompson and colleagues have pointed out²⁵ that the neural hypothesis will not explain what is known about female carriers of the gene for Duchenne muscular dystrophy. According to the Lyon hypothesis, half of the neurones of these carriers will contain a single active X chromosome carrying the dystrophic gene, and half the normal gene. The creatine kinase level in the blood of a carrier might therefore be expected to be half of the level in a male with Duchenne muscular dystrophy of the same age if the neurone caused the skeletal muscle breakdown. This prediction is not substantiated, the values of creatine kinase being much lower.

The state of play may be summarised as follows. Much of the original basis of the neural theory has been contradicted, but neural abnormalities both of structure and function have been found in murine muscular dystrophy. The muscle degeneration in the latter may be of extramuscular origin though the chimaera experiment needs further study. On the whole I consider that there is little incontrovertible direct evidence for the neural hypothesis and that it remains likely that a primary muscle degeneration occurs in many, if not all, of the muscular dystrophies.

There are reasons to turn attention to membrane abnormalities in the muscular dystrophies. There is some suggestion that the sarcolemma leaks creatine kinase at the earliest stages of Duchenne muscular dystrophy, some months or years before muscle fibre necrosis begins²⁶. Red cell membrane abnormalities have been seen with the scanning electron microscope both in murine²⁷ and human Duchenne²⁸ muscular dystrophy, though preliminary studies in my laboratory have failed to confirm acanthocytosis in murine muscular dystrophy, nor demonstrate abnormalities of plasma lipoproteins. It would, however, clearly be interesting to study the other muscular dystrophies with the techniques which have enabled Roses, Appel and colleagues to identify biochemical abnormalities in the membranes of patients with dystrophia myotonica^{29,30}.

W. G. BRADLEY

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'Hot' hydrogen initiates amino acid production

from D. O. Hall

It is just 21 years since Miller reported his now classic experiments in a paper entitled "A Production of Amino Acids under Possible Primitive Earth Conditions". This non-biological synthesis of amino acids by sparking for a week a gaseous mixture of methane, ammonia, hydrogen and water vapour initiated a new phase of research on the origin of life. Since then an impressive list of many different amino acids, sugars, aromatic hydrocarbons and nucleotides and nucleosides have been synthesised using various energy sources such as sparking, heat, ultraviolet (UV) light, α , β and γ rays, and shock waves. These experiments are well described in Miller and Orgel's recent book *The Origins of Life on the Earth* (Prentice-Hall, New Jersey; 1974).

The sulphur-containing amino acids which are so essential components of proteins have been conspicuous by their absence among the products of all these types of experiments. In 1971, however, Sagan and Khare (*Science*, 173; 417) solved this problem by including H_2S in a UV-irradiated gas mixture which included methane, ammonia, ethane and water; cysteine was produced as one of the six amino acids detected. The H_2S provided the source of sulphur and importantly also provided the means by which UV light at wavelengths below 266 nm could be absorbed as an energy source to catalyse the prebiotic synthesis of organic compounds. These authors calculated that about 200 kg of amino acids could have been produced by UV light absorbed by H_2S per square centimetre on the primitive Earth in 10^9 years. This large amount of amino acids must have allowed a high concentration to accumulate even allowing for considerable destruction by other processes.

Hong, Hong and Becker (*Science*, 184, 984; 1974) have now confirmed and extended this earlier work by showing that H_2S or CH_3SH (methyl mercaptan) can act as UV photon absorbers and can initiate by releasing 'hot' hydrogen atoms the production of up to nine amino acids, including the important cysteine. Whereas Sagan and Khare reported that a two-carbon substrate such as ethane is required to produce amino acids, Hong *et al.*'s experiments worked well with just the one-carbon methane. A simple mixture of H_2S , NH_3 , CH_4 and H_2O will produce nine amino acids but interestingly

cysteine may require a two-carbon substrate in order to be synthesised. These authors state that the amino acids produced were in the free form and were not breakdown products of polymers which would have formed as polypeptides—as was suggested by Sagan and Khare. These differences in experimental procedure and products may be important in future 'prebiotic experiments' where the aim is to synthesise polypeptides.

The 'hot' hydrogen atoms produced by the action of ultraviolet light on H_2S or CH_3SH have kinetic energies of about 17 to 32 kcal mol^{-1} , which is sufficient to provide the activation energy for the synthesis of organic compounds in interstellar space or on the primitive Earth. That these types of syntheses may occur in interstellar space has become a real possibility with the recent discoveries using radio astronomy, of compounds such as ammonia, hydrogen, hydrogen cyanide, acetaldehyde, methyl alcohol and hydrogen sulphide in interstellar clouds. In 1971 Buhl reviewed this rapidly developing field (*Nature*, 234, 332; 1971) and concluded with this fine paragraph: "The important parallels appear to be between the formation of interstellar molecules and the synthesis of amino acids in prebiological chemistry experiments. It is here that the key to the evolution of interstellar molecular clouds lies".

Limited progress at GR7

from P. C. W. Davies

ALTHOUGH the location of the Seventh International Conference on Gravitation and General Relativity at Tel Aviv University from June 24–28, was somewhat controversial—few Eastern European countries were represented and some eminent Western relativists were also absent—nevertheless, there was a generous sprinkling of familiar names among the participants and the usual atmosphere of conviviality seemed unimpaired.

On the theoretical side, only modest progress seems to have been made since the sixth conference in Copenhagen. Both S. Deser (Brandeis University) and J. Goldberg (Syracuse University) made discouraging remarks about quantum gravity, reinforcing the prevailing opinion that traditional covariant quantisation techniques are going round in circles, while only hinting at the possible direction of more radical approaches. At a less sophisticated level, the recent claim by Hawking that quantum field theory in a classical gravitational field predicts the disinte-

gration of microscopic black holes seemed to have been readily accepted in spite of the rather unusual nature of the result. (Hawking himself was not present.) The effect was discussed at some length by W. Press (California Institute of Technology) in a summary of astrophysical processes occurring near black holes. Helpful review talks on cosmology and exact solutions were delivered by R. Matzner (University of Texas) and W. Kinnersley (University of Montana) respectively, and a sparkling account of exotic mathematical developments connected with the BMS group was given with much humour by E. Newman (University of Pittsburgh).

The experimental results were rather more dramatic. M. Rees (University of Cambridge) gave a spirited and carefully balanced review of the theoretical and observational aspects of the detection of black holes, concentrating particularly on the situation regarding the X-ray source Cygnus X-1. Having presented some evidence to suggest that this source has a mass of at least $6M_\odot$, he commented that explanations of this object involving models of differentially rotating white dwarfs or three-body systems seemed "contrived and *ad hoc*", and that in his opinion Cygnus X-1 was instead a "strong candidate" for a black hole. Characteristically, his optimism was tempered by the remark that this was "not a firm conclusion", but that the balance of evidence did seem to favour the black hole explanation. If this was so, then there was a new opportunity to test general relativity by examining the behaviour of the accretion disk which would surround such an object and produce the X rays, possibly by relating this behaviour to curious fluctuations in intensity which have been observed from this source.

By far the most controversial issue of the conference was left to the last day, with a panel discussion on gravitational wave detection. During the past few months the interpretation of J. Weber's epic experimental data in terms of gravitational pulses from the galactic centre has been increasingly open to doubt. The subject reached a new peak of controversy with the presentation of results from other similar experiments being carried out in Munich/Frascati, Glasgow and the Bell Telephone Laboratories/University of Rochester. First, Weber (University of Maryland) reasserted his existing position in confident and ebullient style, claiming a further high event rate for the period May 21–June 15, 1974 using improved equipment. He did admit, however, that the use of a new algorithm favoured by other groups led to a greatly diminished event rate, but asserted that his original algorithm had a very much better detection efficiency.

P. Kafka (Max Planck Institute,

Munich) described the Munich/Frascati data since August 1973, claiming a substantial improvement on sensitivity over Weber, but with no significant events detected. R. Drever described data from his wide bandwidth detector in Glasgow during the period September 1972–April 1973 with the same result—no effect. He described the situation as “saddening” and went as far as to express the opinion that Weber was probably mistaken. Finally, J. Tyson (Bell Telephone), claiming a sensitivity for the Bell Laboratories/Rochester experiment very much greater than other experiments, also reported no events.

In view of the importance of the issue at stake, the atmosphere remained remarkably restrained. Weber countered the negative results of his colleagues by rejecting their claims of higher sensitivity. When challenged on the all-important issues of the sidereal anisotropy (which seems to indicate a correlation of his events with the centre of the galaxy), Weber seemed to be saying that he had not yet checked recent data for this effect because of other pressures. The overall impression was that the issues involved hinge on complicated technical details, but that Weber's position among the experimentalists is now growing increasingly isolated as more and more experiments fail to confirm his data.

Stranded whales in Britain

from our
Marine Vertebrate Correspondent

THE gathering of data on stranded cetaceans on the coast of the British Isles has now been in progress for well over half a century. Because of the special historical claim that the Crown had over ‘royal fishes’ on much of the English, Welsh and Scottish coasts, the collection of reports of strandings was already in progress and their publication on a regular basis was a logical step which was initiated by the late Sir Sidney Harmer in 1913.

The latest *Report on Cetacea stranded on the British Coasts from 1948 to 1966* compiled by Fraser (No. 14, 1–65, 9 maps; British Museum (Natural History) 1974, £3.00) contains a full listing of the reported stranded cetaceans (with notes on a few sightings) on the British and Irish coasts for the eighteen year period to 1966. It is unfortunate that even the most recent records in the report are now eight years old, and that the opportunity has not been taken to bring the list as nearly up to date as possible. Nevertheless, there is much of interest in the accumulated records despite their age, and there is good reason

in bringing them all together in the hope that common patterns will emerge from the data.

Among the records of especial interest is the full documentation of two strandings of narwhal (*Monodon monoceros*), both in 1949 (February and July), a species known to have been found only three (or possibly four) times before. A pair of narwhals was also reported as sighted off the Orkneys in June 1949. The white whale (*Delphinapterus leucas*), only stranded once before since 1913, was sighted on three occasions (1948, 1950 and 1965) off the British Isles, and, as Fraser points out, was also reported off the French coast in December 1948, and in Dutch waters in June 1965 and 1966. The coincidence in some of these dates of sighting and later stranding suggests that the same specimens may have been involved in some cases, but this is not possible to confirm. What may also be significant (although Fraser does not comment on this) is that these two species are both Arctic cetaceans which occurred in unprecedented numbers in 1948–1949 in European waters following a winter of unusual severity in 1947–1948.

Other records of particular interest in Fraser's report are the first recorded occurrence of the pigmy sperm whale (*Kogia breviceps*), off Co. Clare, Ireland, in April 1966, and two unrecorded strandings of the euphrosyne dolphin (*Stenella coeruleoalba*). This species had hitherto only been reported twice (1937 and 1939) in British waters, but the two additional records predate these, one based on an old photograph of 1923, and the other on a skull in the British Museum collection, previously identified as a common dolphin, which was stranded in 1934.

The total number of identified stranding since record keeping began in 1913 now amounts to 1,550. Of these 631 were of the common porpoise (*Phocoena phocoena*), 185 of the bottle-nosed dolphin (*Tursiops truncatus*) and 135 of the common dolphin (*Delphinus delphis*). By analysis of the occurrences of the common porpoise, Fraser has shown that strandings occur most frequently during the period July to October, with small peaks in January to March, and on the central and southern North Sea coast. Fraser cites earlier literature which suggests that there is an annual migration out of the Baltic between November and February, and conjectures that this may be correlated with the summer-time peak of strandings on the East Coast of Britain. The pattern of records which Fraser presents is certainly in keeping with the suggestion that porpoises enter the North Sea mainly in the northern sector and then follow the main current systems southwards

in an anti-clockwise direction. On the other hand, the incidence of strandings seems to be high in each of the major bights round the English and Welsh coasts, and the southern North Sea and the eastern English Channel can be seen as particularly large bights. The explanation for the distribution of porpoise strandings may thus be due to the configuration of the coast line and possibly the presence of shoal water, rather than to purely biological phenomena such as annual migrations of the animals.

Lead accretion in alluvial deposits

from Peter D. Moore
Plant Ecology Correspondent

THE release of undesirable but easily recognised substances into the environment, during such processes as mining and industrial smelting or by the explosion of nuclear devices, does have certain compensations. It can provide datum levels in recently deposited sediments which allow the calculation of accretion rates. For example, Bradbury and Waddington (in *Quaternary Plant Ecology*, edited by Birks and West, Blackwell, Oxford, 289; 1973) have used the abundance of haematite granules in the recent sediments of Shagawa Lake, Minnesota, to determine ediment ages. Haematite grains first appear in the sediments when iron ore mining began in the area in 1889 and reached a peak in 1902 when production was maximal; subsequently they declined gradually until 1951 when there was a sharp decrease corresponding to a decline in mining.

The isotope caesium-137 has been used as a datum in both peats and limnic sediments (for example, Pennington, Cambray and Fisher, *Nature*, **242**, 324; 1973). Fallout reached a maximum in 1963 following the widespread proliferation of nuclear testing above ground. Lee and Tallis (*Nature*, **245**, 216; 1973) have used aerial lead fallout to provide datings of stratified peat deposits in the Southern Pennines.

Davies and Lewin (*Envir. Pollut.*, **6**, 49; 1974) have also studied lead, but this time in alluvial deposits of the River Rheidol. The build-up of alluvium in the meander loops of the river has been mapped or photographed on eight occasions during the past 130 years; this has allowed the successive zones of alluvial soil to be dated fairly accurately. The upper catchment of the Rheidol has been mined for lead since Roman times, but peak activity occurred during the nineteenth century. Davies and Lewin have analysed the zones of alluvial soils in an attempt to determine the effects of these activities

upon the floodplain materials of the river.

Deposits laid down in the period 1845–1886 had the highest concentrations of lead (1,500 p.p.m. dry soil). Following this, in the zone 1886–1904, values dropped to 1,011 p.p.m.; the fall could reflect the 1876 Act of Parliament requiring the cleansing of mine effluents. Mining ceased at about 1900 and the subsequent zones have lower concentrations of lead: 1904–1951, 785 p.p.m.; 1951–1971, 503 p.p.m.; 1972, 368 p.p.m.

These data show that, despite the reworking of older deposits during the formation of meanders, the lead contents of alluvial soils do reflect the contemporary conditions of lead pollution in the river. This technique could be used for the historical studies of alluvial pedogenesis in similar situations but where precise mapping is unavailable. The accumulation of heavy metals in alluvium is also a salutary reminder that the precise drainage conditions which make these soils the recipients of leached and eroded plant nutrients, producing high fertility, also result in the concentration of the toxic materials eroded from the catchment. The advisability of using such alluvial soils for arable agriculture or grazing will depend on conditions of river pollution during the period in which accumulation of the substrate took place.

Evaporation and gas exchange in nature

from P. S. Liss

THE evaporation of water from natural water bodies is largely controlled by aerodynamic processes in the air above the water surface. By contrast, exchange of sparingly soluble gases, such as oxygen, carbon dioxide, nitrogen and the inert gases, across air–water interfaces in the environment is almost entirely dependent on the degree of turbulence in the liquid phase. This fundamental difference leads to very different approaches in the study of evaporation and gas exchange in nature. For instance in order to describe evaporation, sophisticated mathematical treatments of atmospheric turbulence are necessary, whereas it seems that simple laminar models can go a long way towards elucidating air–water exchange rates for dissolved gases.

In order for water molecules to evaporate from a water surface they must have sufficient energy to escape from the liquid. Furthermore, the aerodynamic regime in the air above the interface must be such that the water molecule is prevented from con-

densing back onto the surface. At the 22nd Bat-Sheva seminar, which this year took for its subject “Mechanism of Evaporation and Gas Exchange in Nature” and was held from June 16–30 at the Weizmann Institute of Science, Rehovot, W. H. Brutsaert (Cornell University) presented a detailed mathematical treatment of evaporation and showed how semi-empirical theories of atmospheric turbulence can be used to help solve the differential equations involved. These lectures were complemented by those of C. B. Tanner (University of Wisconsin) who reviewed the practical methods so far used to measure evaporation rates over land surfaces. He illustrated the importance of such studies by pointing out that evaporation represents on average 70% of the total outflow from catchments in the United States. In arid regions the figure may rise as high as 95%. The best estimates of evaporation seem to be those based on measurements of the energetics of the process, especially when these methods are locally calibrated. G. Stanhill (Volcani Center, Bet-Dagan) led a discussion on the various methods of preventing evaporation from water bodies and plants. For open waters, monolayers have been tried, but it is hard to maintain a continuous film at the surface when there is any appreciable wind. Pumping cold water from the bottom of the lake to decrease the temperature of the surface and so reduce evaporation is a novel technique which deserves attention. There has been some success in laboratory experiments in which plant leaves are sprayed with a chemical in order to increase their stomatal resistance and so reduce evapo-transpiration, but such techniques do not seem to work well when applied to crops in the field.

A laminar layer model has been used by W. S. Broecker (Lamont Geological Observatory) to characterise exchange of sparingly soluble gases across the air–sea interface. From measurements of Rn-222 and its parent Ra-226 near the water surface, it is possible to calculate the thickness of the laminar layer at the sea surface. From such measurements, made as part of the GEOSECS programme in the Atlantic and the Pacific, there are now reasonable average values for gas exchange rates in the major oceans. These values can be used to calculate the fluxes of many atmospheric trace constituents (both natural and anthropogenic) across the sea surface. S. Emerson (Swiss Federal Institute for Water Protection) showed how similar techniques have been applied in lakes to obtain rates of CO₂ transfer across the interface. His results indicate that, despite reports to the contrary, dissolved carbon is unlikely to be a limit-

ing factor in algal growth in lakes, because CO₂ can transfer from the atmosphere to the water at a rate fast enough to supply the carbon required for phytoplankton growth.

The molecular structure of liquid water was considered by R. A. Horne (Arthur D. Little Inc.). Of especial importance in the context of the seminar was his analysis of how the properties of the very surface layers of water molecules (vicinal water) differ from those of the bulk liquid. Vicinal water seems to differ from the bulk fluid over a wide range of physical (freezing point, viscosity, thermal expansion) and chemical (solubilities of ionic and non-polar solutes) properties, but its thickness is still a subject of much debate.

Thermal disruption in the asthenosphere

from Peter J. Smith
Geomagnetism Correspondent

THE possibility that nonlinear viscous heating could produce thermal runaways in the Earth's interior was first recognised by Grunfest (*Trans. Soc. Rheol.*, **7**, 195; 1963) who used the heat equations to show that, for one-dimensional plane viscous flow under constant stress in a slab, the temperature and strain rate may become arbitrarily large in finite time under adiabatic or near-adiabatic conditions. Many of the ideas initiated by Grunfest were then later applied by Shaw (see, for example, *J. Petrol.*, **10**, 510; 1969) and Shaw *et al.* (*Bull. geol. Soc. Am.*, **82**, 869; 1971) to specific geological situations such as the rheology of basalt, the relation between Earth tides and magmatism in the Sierra Nevada, and Hawaiian volcanism. More recently, Fujii and Uyeda have been quoted as having used the ideas of both Grunfest and Shaw to explain the size of intrusive dykes and the eruptive history of volcanoes.

But talk of magmatism, volcanism and viscous flow leads naturally to deeper thoughts about the asthenosphere in mind that O. L. Anderson and Perkins (*J. geophys. Res.*, **79**, 2136; 1974) have been looking again, but in a rather different way, at the possibility and implications of thermal runaways in the Earth. They begin by drawing attention to the fact that the heat flow equation applicable to one-dimensional viscous flow at constant stress is the same one-dimensional equation as that used to describe thermal explosions in solids, and then go on to explore the consequences of this connection between two apparently quite different situations. The fact is that the common equation implies fea-

tures common to both processes; and this, in turn, suggests that the known characteristics of one process may be used to predict the properties of the other; for example, solid explosives possess instabilities which, when triggered, produce the explosion. The fact that viscous flow is governed by the same basic heat flow equation then implies that regions of viscous flow in the Earth (for example, the asthenosphere) should also possess inherent instabilities which, if triggered, should produce thermal runaways.

Insofar as thermal runaways in the Earth were proposed by Grunfest more than a decade ago, this prediction by analogy may not seem particularly useful. Fortunately, however, there is more to be gained, for, as Anderson and Perkins point out, thermal explosions have been studied in some detail, and the knowledge obtained thereby may be used to extend the analogy with viscous flow. Pursuing this line of attack and making reasonable assumptions about the properties of the heat flow equation, Anderson and Perkins conclude that the potential for thermal runaways in the asthenosphere does indeed exist and that the time taken for the temperature to rise to the very high values necessary would probably be a few tens of million years after the initiating event.

Once such a thermal event has been triggered, the hotter material will rise towards the lithosphere in what Anderson and Perkins choose to call a surge. Some of the stronger surges would actually reach the bottom of the lithosphere but would probably not penetrate it. Instead, the surging material would spread out horizontally and then sink as it cooled, although completely circular paths would not be produced thereby because any given surge would die out in about the same time as it took to grow. On the other hand, the time scale involved here is tens of million of years, so that regions of high temperature (hot spots) would be expected to persist near the lithosphere–asthenosphere boundary for periods significant even in geological terms.

The geological implications of such a situation are clearly important. For example, although the hot asthenospheric material itself may not penetrate the lithosphere, its presence immediately beneath would facilitate partial melting in the upper mantle and lower crust, and thus crustal igneous activity. The significant point to note here is that zones of partial melting produced in this way would be distributed irregularly and would thus not necessarily bear any regular temporal or spatial relationship to steady-state subduction processes. Acceptance

of surges would therefore mean that it would be no longer necessary to force complex arrangements of igneous activity into patterns dictated by plate tectonic models. The case that Anderson and Perkins have in mind here is the wide extent and complex patterns of Cainozoic igneous activity in the south-western United States. The problems involved in attempting to explain this activity in plate tectonic terms have already been emphasised by several workers; Noble *et al.* (*Bull. geol. Soc. Amer.*, **84**, 1393; 1973), for example, noted that the late Tertiary volcanic field of south central Nevada contains materials generated from different sources or different levels or both.

Finally, if thermal runaways produce surges in the asthenosphere, what triggers them? Thermal explosions in solids are triggered by thermal impulses and, by analogy, asthenospheric 'explosions' could be initiated by friction at the lithosphere–asthenosphere boundary, mantle plumes or changes in activation energy (for example, by dilution with a suitable volatile). Anderson and Perkins, however, also draw attention to other possible triggers which are less directly thermal and more concerned with changing asthenospheric flow patterns. Examples are the break-up of continents and the replacement of subduction zones by transform faults. It is clear, for instance, that the encroachment of the Pacific ridge on Atlantic plate must have profoundly affected flow patterns in the region and thus severely disrupted the asthenospheric thermal gradients.

Models of the Earth

from P. G. Richards

THE classical subjects of theoretical geophysics dominated a symposium held from June 25 to July 5 at Cambridge: no neat and easily solved novelties here, but the staples of mantle convection, dynamo motions in the core, scattering theory, weather prediction and earthquake source mechanisms.

At a session honouring Sir Edward Bullard, who is retiring from the Cambridge chair of Geodesy and Geophysics, F. Gilbert and A. Dzeiwonski (University of California, San Diego and Harvard) described notable new levels of sophistication in their study of the Earth's free oscillations. Several hundred new modes have been identified, and the inversion of 1066 data yields an improved Earth model in which the source mechanisms of two deep focus earthquakes can be retrieved accurately. Their results back

up a controversial claim, made earlier, that the source region experiences compression, beginning about 80 s before the origin time as determined from body wave observations.

Since 1972, when the International Union of Geodesy and Geophysics sponsored its first symposium on mathematical geophysics, there has clearly been progress in the understanding of the Earth's core. M. Chinnery (Lincoln Laboratory, MIT) gave a new formulation for finding static deformations of an Earth model with a compressible fluid core, showing that current debate and major disagreements in the literature on this subject can be resolved by working with bulk properties in the fluid (density, pressure) rather than with particle displacements. D. Crossley (Memorial University, Newfoundland) presented results for the gravitational undertones in a subadiabatic core of a rotating Earth, finding that eigenperiods are confined to the range 0–12 h. The written versions of these two papers are awaited with interest, for the basic problem has challenged Love, Jeffreys, Pekeris, and many another of the sharpest theoreticians in geophysics.

In weather prediction and seismic wave propagation, it is now possible to recognise statistical elements which limit the deterministic results naively hoped for a few years ago. However well an initial state of the atmosphere is known, C. Leith (National Center for Atmospheric Research, Boulder) reported, there is no basis for predicting atmospheric pressures beyond about two weeks. Likewise, the seismologist should not expect to interpret every detail on a seismogram, although K. Aki (MIT) has used a standard back-scattering theory to determine some interesting differences in the heterogeneity of California, Japan and Norway. Subsequent corridor discussion showed that scattering theories are being widely investigated, as, for different length scales of heterogeneity, completely different scattering modes may be appropriate. Even the standard formulations (based on acoustic waves) may give different asymptotic results for P–S scattering in the elastic case.

The effect of specified inhomogeneity on sufficiently long period waves should, of course, be deterministic, and quite realistic problems are now often attacked with the finite element method. A major computational advance here was reported by W. D. Smith (University of California, Berkeley), who has found a subtle way to eliminate unwanted reflections from the grid boundaries. He uses two sets of boundary conditions for which the sum of solutions sees the boundary as transparent, even for elastic (P–SV) waves.

Fossil marginal basin in the southern Andes

Ian W. D. Dalziel*, Maarten J. de Wit & Keith F. Palmer†

Lamont-Doherty Geological Observatory of Columbia University, Palisades, New York 10964

In the southern part of the South American continent a marginal basin opened behind an active andesitic island arc in the earliest Cretaceous. The basin closed again in the middle Cretaceous, during the period of fast seafloor spreading, causing the penetrative deformation of the southern Andean Cordillera.

THE Andes are widely regarded as the 'type' continental margin orogenic belt¹⁻⁴. The regular Cordillera-bounded, western continental margin of South America is frequently contrasted with the island arc festooned eastern margin of Asia^{5,6}. Our recent field work demonstrates that a marginal basin with a mafic floor, like those behind the island arcs of the western Pacific, opened up in the southern Andes during the Cretaceous as previously suggested by Katz⁷. This has important consequences not only for the history of the Pacific Ocean basin but also for concepts of mountain building at continental margins.

A narrow discontinuous belt of mafic igneous rocks extends along the spine of the Andean Cordillera from Cape Horn at 56°S at least as far north as 51°S, a distance of 350 km (Fig. 1). These rocks, commonly termed the *rocas verdes* (green rocks) by Chilean geologists, were considered to be mainly lavas and have been variously referred to as 'eugeosynclinal' or 'ophiolite' assemblages⁷⁻¹¹.

The belt of mafic rocks is flanked on the Pacific side by the Patagonian batholith, a vast continuous granodioritic body extending from Cape Horn to 50°S and beyond, averaging more than 60 km in width and reaching almost to the continental margin. The continental rocks on the Atlantic side consist of Palaeozoic (? and older) metamorphics unconformably overlain by an extensive silicic volcanic layer 0–2 km thick of middle–late Jurassic age, with a cover of Cretaceous and Cainozoic sediments. The silicic volcanic layer extends in the subsurface as far as the eastern continental margin.

The Patagonian batholith is interpreted as the root of an andesitic volcanic chain initiated in the Late Jurassic and comparable with the Central Andes of today. The *rocas verdes* are thought to represent the mafic floor of a marginal basin that formed in the latest Jurassic–earliest Cretaceous, separating the active andesitic volcanic chain from stable continental South America.

Volcanic chain development

A thick sequence of sediments was deposited in the southern Andes from Kimmeridgian to Aptian time. Along the Atlantic side of the High Cordillera uniform black mudstones of shelf facies were deposited, thickening markedly from 400 m in the east to more than 2 km towards the west. Within the Cordillera a marked facies and a further change in thickness occurs. Abundant, thick greywacke beds and thin radiolarian cherts appear interbedded with the black mudstones. Towards the Pacific where the sequence exceeds 3 km, the greywackes thicken and become more abundant¹². They are rich in immature

andesitic volcanic detritus, which suggests the presence of an active volcanic source during sedimentation. Variations in thickness and facies indicate that the source lay on the Pacific side of the sedimentary basin. The presence of andesitic lavas and volcanic breccias in the southern part of the basin, interbedded with the sediments but geographically restricted to the Pacific margin, supports this interpretation¹³ (see Fig. 2b).

The Patagonian batholith now lies directly along the Pacific margin of the sedimentary basin. It is a composite,

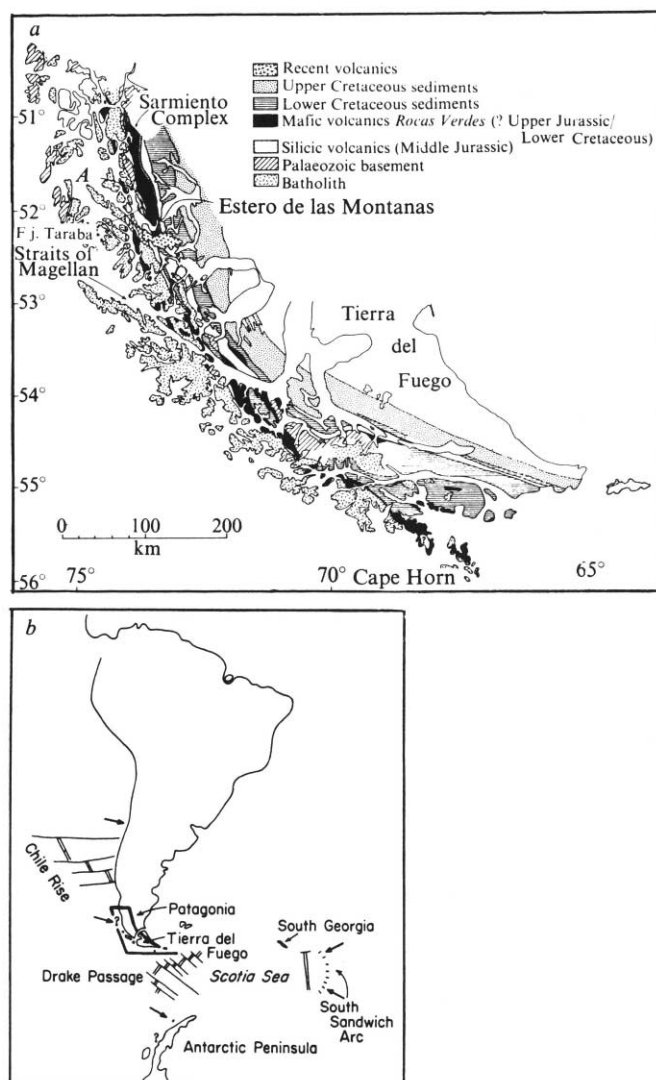


Fig. 1 a, Simplified geological map of the southern Andes, A = Isla Young. b, Location map, arrows show general subduction directions.

and as yet poorly mapped unit consisting of numerous small plutons of coarse grained 'granitic' rocks ranging from tonalite to adamellite. The batholith is intruded into and contains large xenoliths of Palaeozoic metasediments and Late Jurassic silicic volcanics, both readily recognisable as

*Also of Department of Geological Sciences, Columbia University.

†Present address: Churchill College, Cambridge, UK.

parts of the South American continental 'basement' that underlies the Cretaceous shelf sediments to the east (Figs 1 and 2). The Patagonian batholith is strikingly similar to the Peruvian batholith recently described by Cobbing and Pitcher¹⁴.

Hamilton¹⁵ has proposed that large batholiths of intermediate composition, such as those that are more or less continuous along the western North and South American continental margins, are the plutonic equivalents of overlying contemporaneous calc-alkaline volcanic chains. In the southern Andes there is strong support for such a comagmatic origin. Radiometric dating, largely by Rb-Sr whole-rock-mineral isochrons¹⁶, indicates that the batholith was initiated by the Late Jurassic (150 Myr) and that plutonic activity continued intermittently until at least the end of the Cretaceous. Thus it was coeval with the andesitic volcanic activity on the same (Pacific) side of the sedimentary basin.

Thus we conclude that the Patagonian batholith was overlain by an andesitic complex emplaced into South American continental crust, as in the present central Andes, and supplying detritus to the basin on its eastern flank. This then suggests that subduction of the floor of the Pacific Ocean beneath this part of South America was initiated by the late Jurassic.

Marginal basin development

The floor of the basin consists of large lenses (up to at least 120 km long and 20 km wide and more than 2,000

overlap on to these blocks, usually being separated from them by a variable sequence of clastic (often volcanoclastic) sediments including conglomerates, mafic and silicic tuffs, and lavas. The extrusives dip moderately inwards (Fig. 2a). Thus the mafic lenses are funnel shaped in cross section, have normal igneous contacts and are autochthonous.

The sheeted dyke complexes are entirely composed of dykes with chilled margins against each other and basaltic-doleritic textures. They are as much as tens of metres thick but average 1–2 m. Where studied in detail (north of the Straits of Magellan where the Andies trend north-south) they strike consistently subparallel to the Cordillera. Swarms of mafic dykes and gabbro sills are present even outside the main mafic complexes, cutting the rocks of the continental blocks. Where large volumes of these intrusives are present in the continental blocks, especially near the margins of the mafic lenses, remelting and assimilation of the older silicic material is frequently evident. This process has almost certainly resulted in the generation of some of the more silicic phases associated with the gabbros.

Although the original igneous textures are generally preserved in the mafic rocks, the latter generally display zeolite facies mineral assemblages increasing with depth to lower amphibolite facies. At present these assemblages seem to represent an 'ocean floor' type of metamorphism.

The mafic lens we have studied in most detail forms Cordillera Sarmiento north of the Straits of Magellan (Fig. 1). Here the extrusive unit is conformably overlain by fossiliferous sediments dated as Lower Cretaceous (A. Cañon, personal communication). The absence of extensive

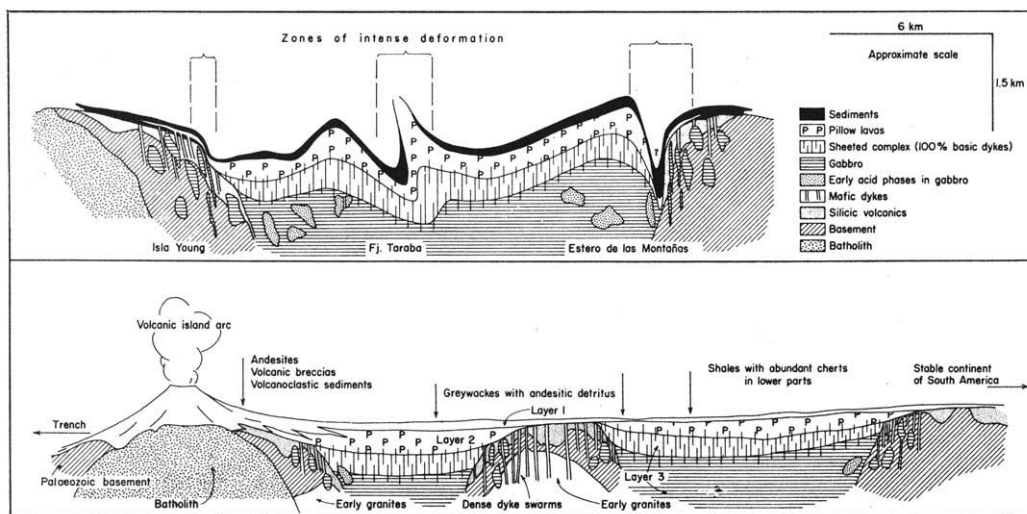


Fig. 2 a, Composite east-west section across the Sarmiento complex (for location see Fig. 1) b, Restored diagrammatic section across the southern Andes in the early Cretaceous.

m thick) of mafic rocks (Fig. 1). The lenses are subparallel to the Cordillera. They intrude and separate blocks of Palaeozoic schist and the overlying Middle-Upper Jurassic silicic volcanics (Fig. 2a).

The mafic rocks represent the upper parts of ophiolite assemblages. An intrusive unit, consisting of gabbro that may show cumulative layering, is cut by, and passes upwards into, a sheeted dyke complex. At still higher levels the sheeted complex is overlain by an extrusive unit of considerable thickness consisting of near horizontal pillow lavas, pillow breccias and tuffs. The intrusive-extrusive transition is complex, the dykes thinning and becoming irregular and more widely separated by screens of pillow lavas. Individual dykes frequently feed pillows. The extrusives are in turn overlain by, and intercalated with, minor amounts of chert and jasper.

The intrusive unit has steeply dipping igneous contacts with the continental blocks. In places the extrusive units

flyschlike sediment layers within and below the mafic extrusives suggests that the latter were extruded very rapidly during a major pulse of magmatism in the early Cretaceous and that marginal basin formation was initiated during the evolution of the andesitic volcanic complex along the Pacific continental margin.

The floor of the marginal basin consisted of huge strips of 'oceanic' crust separated by elongate blocks of older continental crust and must have formed by extension and intrusion of basaltic magma behind an active andesitic volcanic chain. Extension moved the volcanic chain towards the Pacific, relative to South America, producing a volcanic island arc based on continental crust and separated from the stable continent by a marginal basin whose floor had geophysical characteristics partly 'oceanic' and partly 'continental'. The geotectonic setting must have been analogous to the present Japan Sea area in these respects. The apparent absence of ultramafic complexes

from the otherwise complete ophiolite suites of the southern Andes may be the result of structural level, because in Cordillera Sarmiento the gabbros are at sea level. The question of whether or not the crust of marginal basins is identical to that of large ocean basins remains, however, to be settled.

Closure of marginal basin

The youngest dated rocks of the basin infilling are Aptian. The whole sedimentary pile, together with the underlying basin floor, shows signs of strong deformation. These tectonic structures are cut by undeformed granodioritic plutons that have been dated as 80–90 Myr old^{16,17}. It is clear, therefore, that the marginal basin rocks were deformed after the Aptian and before the end of Coniacian¹⁸. We consider this deformation to have occurred when the volcanic arc was translated back towards the continental margin. The resultant shortening between the arc and continent caused the deformation not only of the rocks of the marginal basin but also of its margins, particularly the eastern continental margin.

The very varied basin floor assemblages were very inhomogeneously deformed. Zones of intense and locally polyphase deformation alternate with large areas of virtually undeformed rocks across the width of the basin, the areas of high strain often being concentrated along major lithology contrasts. North of the Straits of Magellan the floor rocks of the basin display essentially vertical planar structural elements (shear zones, slaty cleavage and major fold axial surfaces) that parallel the strike of the Cordillera.

By contrast the sedimentary fill of the basin appears to be more uniformly deformed. A persistent, well developed, slaty cleavage that is axial planar to related asymmetric folds occurs throughout the cover sediments, striking parallel to the Cordillera. South of the Straits of Magellan the cleavage and fold axial surfaces dip towards the Pacific. The vergence of the folds towards the Atlantic^{19,20} is accentuated by small thrusts in the anticlinal hinges that translate the rocks in the same direction. This fold vergence is also predominant in southern Tierra del Fuego where the Cordillera trends west-east^{12,19}. Moreover, if recent reconstructions of the North Scotia Ridge are correct^{21,22}, the flyschlike Cumberland Bay and Sandebugten sequences of South Georgia^{23,24} also represent basin fill, and the Cumberland Bay has the same Pacific to Atlantic vergence^{23,24} and is thrust towards the Atlantic over the Sandebugten sequence that has predominantly opposed vergence²⁵.

The rocks to the east of the marginal basin on the South American continental margin of the early Cretaceous show varying intensity of deformation. Along the whole length of the Cordillera the silicic volcanic layer beneath the Cretaceous and Cainozoic sedimentary cover has been structurally uplifted and crops out in a narrow belt along the Atlantic margin of the High Cordillera. In the north deformation of the Palaeozoic basement schists, like that of the mafic lenses, is confined to narrow zones²⁰. In the east–west trending Cordillera in Tierra del Fuego, by contrast, widespread reactivation of the Palaeozoic basement has occurred^{20,26}. Everywhere the intensity of deformation dies away rapidly towards the Atlantic.

The rocks of the volcanic arc itself were also probably deformed at this time. Some narrow subvertical shear zones in the batholith may be related to basin closure. To the north of the Straits of Magellan the Palaeozoic schists into which the batholith was emplaced are deformed in zones as on the eastern margin of the basin.

The basin must have closed by obduction, subduction or internal deformation of the basin floor and its infill, or by a combination of these processes. As the rocks of the basin

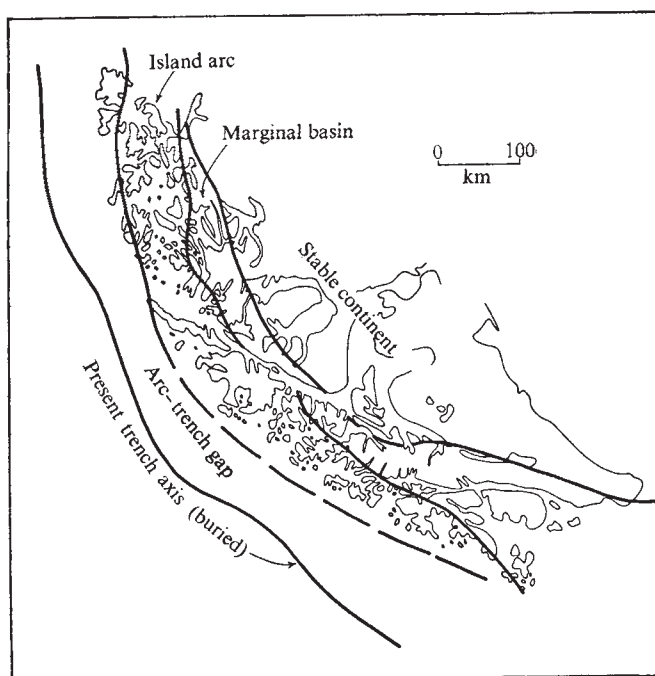


Fig. 3 Present location of the main tectonic divisions of the southern Andes, as interpreted in this paper.

floor appear to be entirely autochthonous, obduction can be rejected. The marginal basin rocks we have studied were certainly horizontally shortened by internal strain. It has not yet been determined whether this resulted from subduction of the basin floor. If subduction occurred the mafic lenses now preserved merely represent relics of the easternmost part of an originally more extensive marginal basin.

Geotectonic implications

Several significant conclusions can be drawn:

(1) There is positive evidence in the southern Andes that the Mesozoic batholith along the Pacific continental margin represents the root of an andesitic volcanic chain that was initiated on South American continental crust by the late Jurassic. We relate the formation of the volcanic chain and its continued activity into the Cretaceous to subduction of the Pacific Ocean floor beneath the South American continent.

(2) In the latest Jurassic–early Cretaceous, basaltic magmas were intruded into the continental crust behind the volcanic complex that was consequently translated towards the Pacific relative to South America, creating a marginal basin between a volcanic island arc and the South American continent. The marginal basin seems to have formed during a single major pulse of rapid extension, and was infilled during the early Cretaceous by andesitic detritus from the active arc. The original width of the basin is not known.

(3) During the middle Cretaceous the arc moved back towards the continent accompanied by the deformation of the basin rocks, the South American continental margin and probably the arc itself. Recognition of the inhomogeneous deformation of the mafic lenses leads us to emphasise that the dating of ocean floor remnants solely on the basis of the presence or absence of deformation fabrics such as has been attempted in the Appalachian/Caledonian orogen^{27,28} must be undertaken with the greatest caution (J. R. Burnsall and M. J. de Wit, in preparation).

(4) Based on fossils in the sediments overlying the mafic

lenses, it can be interpreted that the marginal basin opened before 118 Myr (base of the Barremian). From the age of the plutons cutting the deformed basin infill, it closed before 88 Myr (base of the Coniacian). Thus whereas the opening took place during a period of 'normal' seafloor spreading rates in the Pacific, closure and accompanying deformation coincided with the period of particularly high spreading rates between 110 and 85 Myr ago²⁹.

The opening of the marginal basin took place by extension and intrusion behind the arc, apparently in the manner suggested by Karig³⁰ and Uyeda³¹. Field observations clearly demonstrate that the 'ophiolitic' rocks do not represent old oceanic crust fortuitously trapped between South America and an island arc migrating across the Pacific^{2,6}. Closure of the basin did not take place by obduction of the basin floor. Horizontal shortening was certainly involved and Pacific-ward subduction may have taken place.

In the Central Andes and in West Antarctica ophiolitic rocks are unknown^{10,14,22}, although there is substantial evidence of Mesozoic subduction^{32,33}. Hence it seems that a marginal basin with a mafic or partially mafic floor did not develop in these areas. At least in the central Andes, however, an early Cretaceous sedimentary basin infilled with flysch-type sediments full of andesitic detritus and mafic extrusives, did develop behind the Mesozoic volcanic chain, perhaps indicating more limited extension. Significantly, the mid-Cretaceous deformation there was far less intense and of a different style, consisting of block faulting and gentle warping. Penetrative fabrics are wholly absent. Thus it seems that large penetrative strains at high crustal levels are not generated behind a volcanic arc by subduction alone but may be related to 'collision' between volcanic arcs and continents during closing of marginal basins.

The data show that marginal basins are not characteristic solely of the western side of the Pacific Ocean. They have existed in the past along the eastern margin. Hence the present western South American continental margin is not representative of all Mesozoic-Cainozoic Andean geological history. Recognition of this fact leads us to consider the factors involved in the opening and closing of marginal basins.

Wilson and Burke⁵ have suggested that marginal basin formation is related to the relative motion of lithospheric plates and the mantle underlying the asthenosphere. According to their hypothesis the consuming plate at a subduction zone, such as the American plate at the Peru-Chile trench, if advancing over the lower mantle, will develop no marginal basin. If, however, the over-riding plate is stationary over the mantle or retreating from the plate being consumed, then marginal basins will develop as in the western Pacific.

Scholz *et al.*³⁴ have suggested a more general explanation that marginal basin formation is related to the level of the horizontal component of compressive stress across the consuming plate boundary. An increase in this component could arise from an increase in spreading rate and it may not be a coincidence that the age of closure of the marginal basin in the southern Andes coincides with the world-wide pulse of high spreading rates²⁹. In addition to changes in relative motion of plates over the mantle, increase in spreading rate and certain changes in plate configuration, it has been suggested that marginal basin closure could also result from weakening of the mafic floor of the basin because of high heat flow below an insulating 'blanket' of volcanoclastic sediments. The metamorphic assemblages of the southern Andean mafic lenses could be taken to support this view but the fact that a uniform penetrative fabric is lacking seems to argue against it.

The question of why the basins open and close is at present unresolved, and it is increasingly apparent how crucial the answer is for understanding the evolution of continental margins and mountain belts. Recognition not only that a marginal basin developed in the Andes, but that it opened and closed before those of the present western Pacific were initiated, may be of great significance for understanding of the evolution of continental margin orogenic belts and the history of the Pacific Ocean basin.

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Crystallographic study of the interaction of urea with lysozyme

K. W. Snape, Robert Tjian*, C. C. F. Blake & D. E. Koshland, jun.†

Laboratory of Molecular Biophysics, Department of Zoology, South Parks Road, Oxford OX1 3PS, UK

High-quality crystals suitable for an X-ray crystallographic study have been obtained for urea-denatured lysozyme

As more protein structures become known through the use of crystallographic methods, knowledge of the basic structure, and also of perturbations of the structure will be required to understand the forces involved in the architecture of the proteins and the catalytic power of enzymes. One of the perturbations which may prove useful, is the unfolding of proteins caused by high concentrations of urea. Although many solution probes such as nuclear magnetic resonance (NMR), electron spin resonance (ESR) and fluorescence, have been used in denaturation studies, these tools, powerful as they are, can probe only small portions of the protein at a time. Such a partial picture makes it difficult to relate structure change with activity since only some of the changes are known. A comprehensive recording of all changes could be obtained by crystallography but unfortunately crystallisation of proteins in urea has seemed difficult theoretically or recalcitrant experimentally. It was therefore decided to explore conditions for obtaining enzyme crystals from urea by the manipulation of urea and salt concentrations. This has now been achieved for lysozyme and excellent X-ray diffraction patterns have been obtained from crystals in equilibrium with high urea concentrations.

Preparation of lysozyme crystals

Native, tetragonal crystals of hen egg white lysozyme chloride were grown following the method of Alderton and Fevold¹: 400 mg of lysozyme were dissolved with thorough stirring in 5 ml of 0.04 M acetate buffer pH 4.7 and the stirring was continued for a further hour to dissolve nuclei. Sodium chloride solution (5 ml, 10% w/v) was added dropwise over several minutes, the final solution was filtered into a polythene bottle and left undisturbed for up to two weeks. Crystal nucleation generally occurred within a few hours but growth was regarded as complete only after two weeks had elapsed.

Tetragonal crystals of lysozyme were crystallised with, for example, 6 M urea by a simple variation of the above procedure: 300 mg of lysozyme were dissolved in 2 ml of a solution which was 4 M in acetate buffer pH 4.7, and 9 M in urea. After equilibrium (assumed to be up to 1 h), 1 ml of saturated sodium chloride solution was added and after further gentle stirring the solution was filtered and left. Nucleation and growth of the crystals was generally as rapid as for the native crystals.

Equilibration of native lysozyme crystals with urea solutions was achieved by first stabilising the crystals with a solution of 0.2 M acetate buffer, 12.3% w/v NaCl and subsequently with solutions of the same salt content but

with the appropriate urea concentration. Equilibration was achieved by adding the final stabilising solution dropwise over several days, with gentle agitation at each addition, to the crystals in their original mother liquor.

Hen egg white lysozyme (3X-crystallised, dialysed and lyophilised), Lot 70C 8110, and N-acetyl glucosamine (NAG) (Sigma (London) Chemical Co.), Tri-N-acetylchitotriose (tri-NAG) and analytical grades of urea, glacial acetic acid, sodium acetate trihydrate and sodium chloride

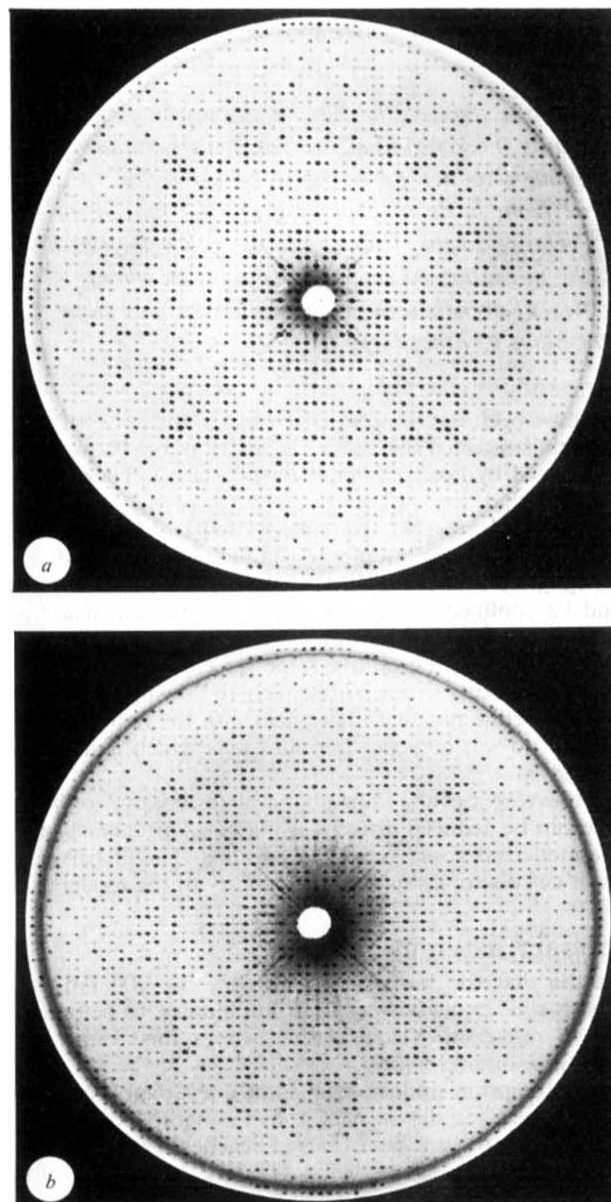


Fig. 1 18° HKO (2.5 Å) precession photographs of tetragonal crystals of hen egg white lysozyme chloride: *a*, native crystals; *b*, crystals equilibrated with 9 M urea.

Present addresses

*Department of Biological Sciences, Harvard University, Cambridge, Massachusetts 02138.

†Department of Biochemistry, University of California, Berkeley, California 94720.

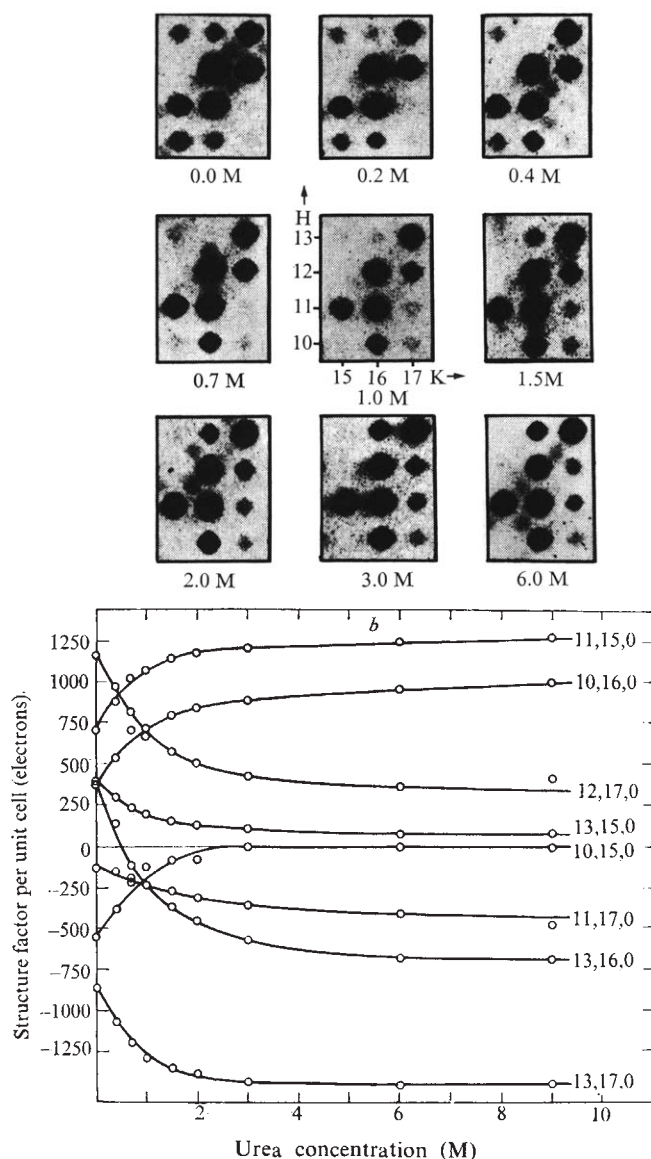


Fig. 2 *a*, Enlargement of a representative group of medium resolution HKO reflections showing the variation of intensities with urea molarity. *b*, A plot of the variations with urea concentration of the structure factors of some of the best-phased reflections of *a*.

(British Drug Houses, Ltd) were used. The urea was freshly recrystallised from glass distilled water immediately before use and only freshly prepared urea solutions were used. Screened precession photographs (18°) of the (centric) HKO and HOL zones were obtained using Ni-filtered copper radiation from an Elliott Automation rotating anode, Type GX3. Photographic intensities were estimated using a Joyce-Loebl double-beam recording microdensitometer Mark III CS. Crystals were conventionally mounted and sealed in contact with the vapours of their respective equilibrium soaking solutions.

Isomorphism of crystals

Crystals of hen egg white lysozyme in equilibrium with solutions of urea show diffraction patterns indicating isomorphism with native crystals². Significant alterations in the intensities of reflections were observed without any loss of order in the crystals or any large change in cell dimensions (compare Fig. 1). The detailed X-ray crystallography therefore confirmed the qualitative observation that good crystals existed in the urea plus salt mixture and that the

crystals give X-ray patterns of the same quality as do the native crystals.

Examination of the photographs (of which Figs 1 and 3 include two representative examples) shows large intensity changes in many of the reflections. These intensity changes indicate that urea causes extensive structural alterations without any resultant loss of crystallinity. This clearly allows a structural investigation of the lysozyme molecule in high concentrations of urea to be carried out using X-ray techniques. Extensive studies of lysozyme structure and activity in urea solutions have been made³⁻¹¹, which have indicated that the lysozyme structure is generally rather resistant to denaturation by urea but that changes do occur. X-ray crystallography confirms these findings in general but allows more specific conclusions to be made. Edelhoch and Steiner⁵, for example, found that the molecule retained some structure, that is, was not a random coil, even in 9 M urea. The X-ray results are in agreement with this conclusion and indicate a surprising degree of order for a solvent of such denaturing capacity.

The changes in intensity are unfortunately much too great (compare Fig. 1) for a conventional difference synthesis against the known native structure to be useful in defining the precise nature of the changes induced by urea. In these circumstances there is no alternative to a complete *de novo* structure determination of the denatured molecule, and a search for useful heavy-atom derivatives has been started. Some useful information of a general nature can, however, be deduced from the intensity changes. The alterations in intensity are particularly pronounced at high resolution, indicating that changes occur largely to the detailed structure of the molecule. A three-dimensional difference map between the 3 M urea and native forms, using terms to 3 Å resolution, while confirming our judgement that the intensity changes were too extensive to be useful in detail, showed that the major difference density features were confined within the molecular envelope. This indicates that while some contribution to the intensity changes can probably be expected from perturbation of the solvent structure, it is clear that the major contribution arises from conformational changes distributed throughout the molecule itself.

Variation with urea concentration

Preliminary phase information from the heavy-atom search together with an analysis of the variation of X-ray intensities as a function of urea concentration allow some broad but definite inferences to be made about the lysozyme-urea system under study. An examination of precession photographs extending to 2.5 Å resolution shows that the intensities of a majority of the centric HKO and HOL reflections vary in a monotonic manner. The detailed intensity variations of a small number of representative medium-resolution reflections are shown in Fig. 2*a*, and a plot of the structure factors of the best phased reflections in this group in Fig. 2*b*. Figure 2 shows that the major changes in intensity have occurred by the time the concentration of urea has reached 3 M, and that the further changes up to 9 M are small. This suggests that the major conformational changes induced by the urea have occurred at these low concentrations, and that thereafter further alterations are minor.

A small number of reflections seem to behave in a different way. Their intensities first decrease to zero as the urea concentration increases and then subsequently increase at higher concentrations. The preliminary phase information indicates, however, that the reflections behaving in this way have all undergone a change of sign (see, for example, 13, 16, 0 in Fig. 2*a* and *b*) and when this factor is taken into account, all the reflections examined are found to vary monotonically. The finding of such

monotonic changes in the diffraction pattern might indicate a two-state denaturation model, or at least be consistent with such a model. But, the knowledge of protein structure which already exists, and the failure of two-state models to account for the kinetics of either denaturation^{12,13} or refolding^{14,15} suggests that such a model is unlikely, and indeed a plot of fractional structure factor changes as a function of urea concentration for individual reflections gave differing curves indicating deviations from a mutually linear relationship. Additional small intensity changes due to the minor concentration-dependent changes in cell dimensions cannot be corrected for, however, and these data *per se* cannot exclude a two-state model. It is, of course, conceivable that, because we are dealing with the structure in a crystal, there are certain constraining relationships which favour an apparent two-state model. A definitive answer to this problem must await the complete three-dimensional crystallographic study which is now in progress.

Stability of the protein

A particularly interesting feature of this X-ray study is the thermodynamic stability both of the protein and of the tetragonal crystal form. Changes in the diffraction patterns seemed to be complete within 24 h of the introduction of urea, suggesting a rapid attainment of equilibrium, but there were no further changes when the crystals were subsequently exposed to the urea solutions for a period of weeks. Crystals equilibrated with high urea concentrations could, in addition, be leached with urea-free high salt solutions and were found within 24 h to regain in full their original native diffraction patterns. That this stability represents a true thermodynamic minimum was demonstrated by the crystallisation of lysozyme directly from urea solutions. Crystals obtained from 1 M urea had diffraction patterns which were identical to those of soaked crystals, whereas those obtained from 6 M urea showed only minor high-angle differences when compared with their corresponding soaked crystals (compare Fig. 3). This retention of isomorphism is especially interesting in view of the appreciable conformational changes indicated both by physical studies and by the present work, and confirms that the basic form and packing of the molecules is not altered as much as might be expected.

No glutaraldehyde or other cross-linking material has been used to cross-link the crystals. The main feature that has changed the urea from a medium which dissolves protein to one in which crystals are stable is the use of high salt concentrations.

While this work was in progress, we learned of the study of Berthou and Jolles¹⁶ who have also crystallised lysozyme from urea. Their studies on temperature effects and polymorphism should offer additional valuable insights into lysozyme properties.

Further uses

In summary, these studies indicate that crystallisation of proteins from urea is possible and that the X-ray crystallography of these structures will be valuable in interpreting the role of conformation in the activity and folding of proteins. The use of urea for structural changes has considerable advantages in studying protein structure since it is the differences rather than the creation of a total structure that is being dealt with. Furthermore, the use of lower concentrations of urea can in some cases produce smaller perturbations of structure. We hope that further work will relate conformational changes to the activity of the lysozyme. Preliminary (unpublished) results indicate that activity is regained and conformational changes are induced when polysaccharides are added.

X-ray crystallography is particularly valuable for such structure-activity correlations. The examination of any

one group, such as by using reporter groups is useful, but the role of a residue may be ambiguous if the perturbations of other groups are not known. Crystallography gives an overall three-dimensional picture which would be difficult or impossible to achieve with individual probes, but is also more time consuming. Eventually parallel relationships in which the probing of an individual group by a reporter group can be correlated with key X-ray structures may offer the complementary advantages of completeness and economy of effort. Our present X-ray results with lysozyme make possible such interrelated studies.

The generality of the method is supported by unpublished studies on chymotrypsin which have shown that it can be crystallised from 6 M urea and remain isomorphous with the native enzyme. Thus, the results found here in relation to lysozyme can be applied to a variety, possibly a majority, of protein molecules.

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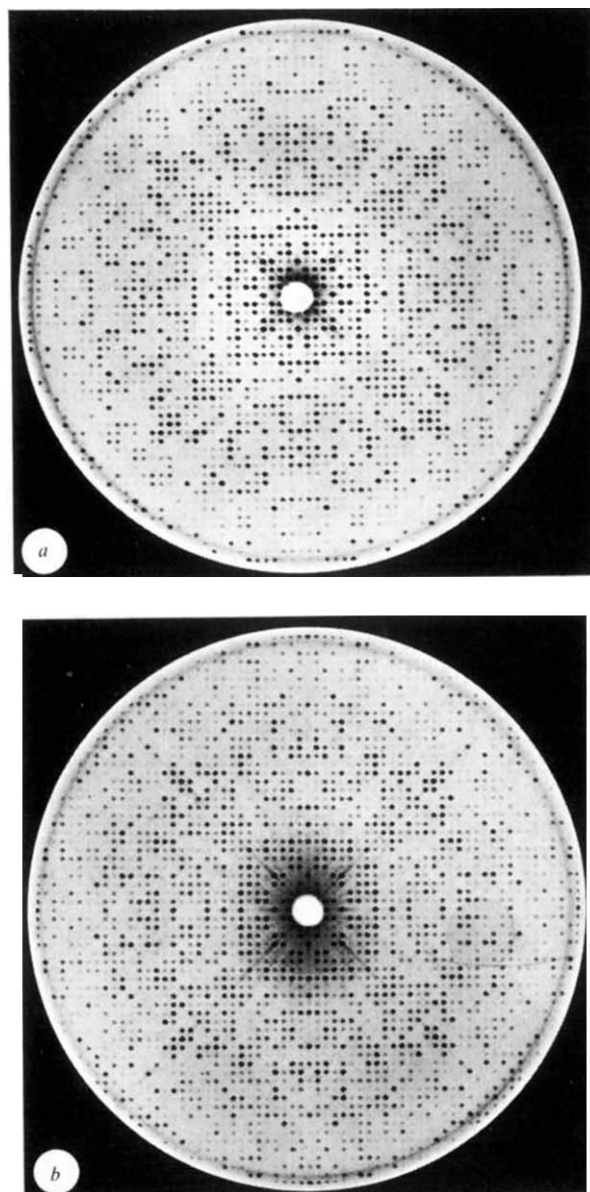


Fig. 3 HKO precession photographs of crystals of hen egg white lysozyme: *a*, soaked in 6 M urea; *b*, cocrystallised from 6 M urea.

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Plate movement relative to rigid lower mantle

L. Lliboutry

Department of Geophysics, Grenoble University I and Laboratoire de Glaciologie du CNRS, Grenoble, France

The vertical extent of the flow in the upper mantle may be governed by the layering of the Earth, with the lower mantle rigid enough to act as a frame of reference. A theorem deduced from basic mechanics gives the movement of the plates relative to this frame. The fit with velocities deduced from the migration of deep volcanism is excellent. Absolute velocities of trenches may be related to the slope of the sinking slabs of lithosphere.

THERE have been several successive steps in the hot spot theory¹. It was first suggested that volcanic chains of the Hawaiian type are produced by hot spots deep in the mantle. Then came the suggestion that the hot spots form a consistent frame of reference. If this were so, then at a particular depth, the bulk of the mantle outside the hot plumes should be subject to shrinking at a negligible rate only. Furthermore, horizontal movements should be confined within the upper mantle and near to the mantle-core interface. The third suggestion was that hot spots could also provide the motive force for continental drift, with horizontal currents in the asthenosphere flowing radially away from each of the plumes. I here deal with the first two suggestions, but do not consider the third idea which is much more controversial.

The movement of the lithospheric plates relative to the lower mantle can be deduced directly, using basic mechanics, as long as the model fits some general conditions. The fit between the relevant calculations supports the validity of the model used.

Necessary assumptions

I assume that the lower mantle, below the 700 km discontinuity, is rigid enough away from hot plumes, to be taken as a frame of reference. Velocities relative to this frame will be termed 'absolute velocities'.

This assumption conflicts with some questionable estimations of the viscosity at depth. McConnell's method of calculating velocities from the profiles of raised beaches² gives no pertinent information about regions below 800 km. Although Goldreich and Toomre³ have explained the nonhydrostatic bulge of the Earth by assuming a mean viscosity $< 10^{24}$ poise, an earlier explanation⁴, which suggested a mean viscosity $\approx 10^{26}$ poise has not been disproved. A mean viscosity of 10^{23} poise at the

mantle-core interface, with a shear strain rate of 10^{-16} s^{-1} has been calculated⁵. It is, however, the shear stress, not the shear strain rate, which is spread over the whole mantle with the same order of magnitude (for instance 5-10 bar), with convection, or without it. With such a shear stress in the asthenosphere, where the melting point is reached. Weertman's law⁶ would give a shear strain rate of 10^{-8} to 10^{-9} s^{-1} , a value 10^8 times too high. By modifying the numerical factor accordingly, the viscosity at the mantle-core interface rises to 10^{27} poise.

My second assumption is that, with the exception of hot plumes and descending slabs, there are no lateral variations in the viscosity of the upper mantle. A three-layer model can be adopted. The top layer is composed of rigid lithospheric plates, which may be limited downwards at an isobaric surface p_0 without substantially changing the dynamical problem. Under this is a low viscosity layer (for instance 4×10^{20} poise). The bottom layer is more viscous (for instance 10^{22} poise), and is of uniform thickness and horizontal extent. The viscosities of layers 2 and 3 are assumed to be Newtonian for the very slow motions involved.

My third assumption is that the viscous layers have densities ρ_1 and ρ_2 , respectively, with horizontal variations which are much larger than the vertical variations within a single layer. The horizontal variations are a function of temperature changes which are governed by the convection processes, and so this assumption implies that any reversal of the current should occur near an interface between layers.

Absolute velocities

Departures from the hydrostatic values are here termed 'perturbations'. The equations of quasistatic equilibrium and of viscosity can be written in a linear form with respect to the perturbations. This is not so, however, with the heat equation, because the convective term in $\mathbf{u} \cdot \nabla T$ is the main one; thus it is not introduced, and the horizontal density gradients $\nabla \rho_1$ and $\nabla \rho_2$ are considered as unknown functions.

I have shown⁶ that, in the two-dimensional case, any perturbation may be expressed as a linear function of four perturbations. With the x -axis horizontal, the velocity u_{ox} at any point on a plate may be written:

$$u_{ox} = C_1 \partial \zeta / \partial x + C_2 \partial p_1 / \partial x + C_3 \partial p_2 / \partial x + C_4 \tau_{2x} \quad (1)$$

where ζ denotes the vertical deflection of the isobaric surface p_0 and τ_{2x} is the shear stress, in the x direction, between the

third layer and the motionless lower mantle. C_i is constant (for a given model) independent of the perturbations. Equation (1) holds to the second order only, but because with reduced variables C_i is of the order of unity and the perturbations are of the order of 10^{-3} – 10^{-4} , this inaccuracy is of little concern.

In the three-dimensional case, equation (1) holds in the x direction, and in a transverse horizontal direction (substituting y for x) because, to the second order, the perturbations in the x and y directions do not interfere with one another. Therefore:

$$\mathbf{u}\partial = C_1\nabla\zeta + C_2\nabla\rho_1 + C_3\nabla\rho_2 + C_4\tau_2 \quad (2)$$

According to my third assumption, equation (2) is valid over the whole Earth with the same constants C_i , with the exception of some small areas (oceanic ridges, subduction zones, and so on). Of course, for points on one plate the values of $\mathbf{u}_0$ are linked and thus the four vectors on the right hand side of the equation are not independent. When crossing a ridge or a subduction zone, ζ does not undergo a discontinuity. The same is true, to the second order, for ρ_1 and ρ_2 , as the conditions are very close to hydrostatic.

(It should be noted that the nullity of the total mass transport through the three layers is not introduced, as was the case in previous articles^{6,7}. Even if there were no hot plumes rising from the lower mantle, this nullity would hold only in the two-dimensional case, that is for a 'cylindrical Earth'. This point will be developed elsewhere.)

There can be a horizontal vector, $\mathbf{a}$, defined at any point on the Earth's surface. The resulting moment of $\mathbf{a}dS$ over the whole Earth, with respect to an arbitrary diameter DD' is

$$\iint (\mathbf{OD} \times \mathbf{OM}) \cdot \mathbf{a} dS = \mathbf{OD} \cdot \iint (\mathbf{OM} \times \mathbf{a}) dS \quad (3)$$

(taking the radius of the Earth, OM , as unity of length). This quantity vanishes when, and only when:

$$\mathcal{M}_0(\mathbf{a}) \equiv \iint (\mathbf{OM} \times \mathbf{a}) dS = 0 \quad (4)$$

It can easily be shown that, when $\mathbf{a}$ is the gradient of a function continuous over the whole Earth, $\mathcal{M}_0(\mathbf{a}) = 0$

Such is the case for ζ , ρ_1 , ρ_2 , and thus:

$$\mathcal{M}_0(\nabla\zeta) = \mathcal{M}_0(\nabla\rho_1) = \mathcal{M}_0(\nabla\rho_2) = 0 \quad (5)$$

Moreover, as the rigid lower mantle is assumed to be spherical, the quasistatic equilibrium of the whole system means that $\int \mathcal{M}_0(\tau_2) = 0$. Thus, according to equation (2)

$$\mathcal{M}_0(\mathbf{u}_0) = \iint (\mathbf{OM} \times \mathbf{u}_0) dS = 0 \quad (6)$$

For any closed path drawn on a single plate, the conservation of mass requires,

$$\oint (\mathbf{OM} \times \mathbf{u}_0) d\mathbf{s} = 0 \quad (7)$$

where $d\mathbf{s}$ is an infinitesimal arc.

As this relationship no longer holds when the path crosses a ridge or a trench, equation (6) is important.

Ω is the instantaneous rotation vector of the plate at point M , and, relative to the lower mantle, $\mathbf{u}_0 = \Omega \times \mathbf{OM}$. Equation (6) can thus be written

$$\iint (\mathbf{OM} \times \Omega \times \mathbf{OM}) dS - \iint \Omega dS \cdot \iint \mathbf{OM} \cdot (\Omega \cdot \mathbf{OM}) dS = 0 \quad (8)$$

Ω is different from plate to plate.

In Cartesian coordinates, with the origin at the Earth's centre O , the components of $\mathbf{OM}$ are x , y , z , and of Ω : Ω_x , Ω_y , Ω_z . Equation (8) becomes:

$$\iint \Omega_x dS - \iint x(x\Omega_x + y\Omega_y + z\Omega_z) dS = \iint (y^2 + z^2) \Omega_z dS - \iint x(y\Omega_y + z\Omega_z) dS = 0 \quad (9)$$

with two other similar relationships obtained with permutations of x , y , and z .

ω of components ω_x , ω_y , ω_z , can be the rotation vector of any plate relative to a reference plate (the Antarctic plate in these computations), and Ω_0 , of components a , b , c can be the 'absolute' rotation vector of the reference plate. Then:

$$\Omega_x = \omega_x + a \quad \Omega_y = \omega_y + b \quad \Omega_z = \omega_z + c \quad (10)$$

As:

$$\iint xy dS = \iint xz dS = 0$$

$$\iint (y^2 + z^2) dS = 8\pi/3 \quad (11)$$

it follows that:

$$a = -3/8\pi \iint (y^2 + z^2) \omega_x dS + 3/8\pi \iint x(y\omega_y + z\omega_z) dS \quad (12)$$

with two similar relationships for b and c , obtained by permuting x , y , and z .

Comparison with former estimations and results

In an earlier publication⁷ the two-dimensional relationship of equation (1) has been extended to cover the whole Earth. Only the plates of the equatorial zone were considered, and the effect of other plates was neglected. That is a very crude model of a 'cylindrical Earth'. This procedure was equivalent to neglecting the second integral on the right hand side of equation (12). If there were a very large number of plates, with random rotation vectors, this second integral should in fact vanish.

McKenzie⁸ has suggested that the term 'polar wandering', which described the motion of the pole relative to a frame of reference, should be defined such that

$$\iint \Omega dS = 0 \quad (13)$$

In other words, he defines 'absolute' velocities of continental drift by:

$$\mathbf{a} = -1/S \iint \omega_x dS \quad (14)$$

To study of the causes of polar wandering it seems preferable to use equation (12) instead. 'Polar wandering' would then be the motion of the pole relative to the bulk of the mantle, excluding its outer viscous shell, that is, relative to the rigid body which carries most of the Earth's momentum. It follows from equation (7) that both definitions would be identical if $\mathbf{OM} \cdot \Omega \approx 0$ for all of the plates. This, however, is not the case.

The computation has been done using Chase's determination of the relative rotation vectors⁹, which takes into account the velocities at the ridges (deduced from the magnetic lineations of the ocean floor) and the direction of the transform faults, but which does not use data on the migration of volcanism (which may then be used as an independent check). To render the integrals discrete, the Earth's surface has been divided into bands of 5° of latitude, and for each band the boundaries between the plates have been located to within 1° of longitude.

Table 1 gives the 'absolute' rotation vector of the Antarctic plate according to McKenzie's definition (equation 14), to the two-dimensional approximation, and to my present theory (equation 12). The differences are not negligible. Table 2 gives the relative rotation vectors calculated from Chase's data, and the absolute rotation vectors according to my present theory.

Table 1 Absolute rotation vector of the Antarctic Plate (10^{-7} degree $^{-1}$ yr $^{-1}$)

	McKenzie's definition	Two-dimensional model	Present theory
a	-0.622	-0.549	-0.207
b	-0.079	-0.223	-0.394
c	2.381	2.539	2.658

Table 2 Relative and absolute* rotation vectors (10^{-7} degree yr^{-1})

Plate and area (Earth's surface = 4π)	ω relative to the Antarctic Plate	Ψ , relative to the Cartesian coordinates†	relative to the lower mantle Spherical coordinates
Antarctic 1.431	$\omega_x = 0$ $\omega_y = 0$ $\omega_z = 0$	$\Omega_x = -0.21$ $\Omega_y = -0.39$ $\Omega_z = 2.66$	$\lambda = 81^\circ$ $\phi = -18^\circ$ $\Omega = 2.70$
American 2.627	0.42 -1.39 -2.96 -1.01	0.22 -1.78 -0.31 -1.21	-10° -83° 1.82 41°
Eurasian 1.689	-0.72 -1.21 -0.98	-1.11 1.45 -1.18	-138° 2.19 -65°
Pacific 2.743	3.20 -9.18 1.64	2.80 -6.52 1.43	113° 7.19 45°
African 1.944	-2.03 0.18	-2.43 2.84	-59° 4.00
Indian 1.637	5.70 2.54 1.79	5.49 2.15 4.45	37° 21° 7.39
Cocos 0.095	-6.90 -15.02 4.74	-7.10 -15.42 7.40	24° -115° 18.52
Nazca 0.400	-1.90 -4.26 3.38	-2.11 -4.65 6.04	50° -114° 7.91

* According to present theory.

† x -axis points towards 0° N, 0° E; y -axis towards 0° N, 90° E; z -axis towards 90° N, 0° E.

It seems that the South American plate, because it has a pole of rotation on itself, and the Antarctic plate, because it has a pole of rotation very near itself, are almost motionless. (Some symmetrical, eastward motions are occurring in Patagonia and the Antarctic Peninsula. These increase towards the Drake Passage.) Africa and the Indian Plate are moving towards the north-east, Europe towards the east, Indochina and Indonesia towards the south-east, and Alaska towards the south. This asymmetry of absolute motions on both sides of the Pacific Ocean may be related to the asymmetry in the slope of the descending slab¹⁰. Any descending slab should have a tendency to sink vertically downwards under its own weight. Nevertheless, if the trench is moving towards the ocean, the horizontal velocity of the trench must be added to the vertical velocity, to give the slope of the slab. This should be the case on the western side of the Pacific Ocean. There, the absolute velocity of the Eurasian plate, and the extension of the marginal seas (which has its own causes), add together to give a large eastward absolute movement of the Japanese-Kuril Trench. Thus, the sinking slab under the Japanese sea has a uniform slope of 30° until its very end, at a depth of 600 km whereas the lower end of the sinking slab under South America is almost vertical.

Comparison with migration of volcanism

If λ_p and ϕ_p are the latitude and longitude, respectively, of a pole of absolute rotation, then its distance, γ , from a point, λ , ϕ , in the corresponding plate is given by the relationship:

$$\cos \gamma = \cos \lambda \cos \lambda_p \cos (\phi - \phi_p) + \sin \lambda \sin \lambda_p \quad (15)$$

The absolute velocity, u_0 , at (λ, ϕ) is then

$$u_0 = (10/9) \Omega \sin \gamma \text{ cm yr}^{-1} \quad (16)$$

where Ω is expressed in 10^{-7} degree yr^{-1}

The azimuth (angle with the meridian) A is given by:

$$\cos A = (\Omega_x \sin \phi - \Omega_y \cos \phi) / \Omega \sin \gamma \quad (17)$$

For the Hawaiian chain, $u_0 = 7.6 \text{ cm yr}^{-1}$, $A = 63^\circ 1/2$, and for the Toubouai Islands (Austral chain), $u_0 = 7.7 \text{ cm yr}^{-1}$, $A = 65^\circ 1/4$. These values may be compared with those deduced from the hot spot theory¹¹. The azimuths are correct to less than 1° , and the velocities are within the limits of confidence.

According to our theory, the absolute motion of the Cocos Plate at the Galapagos Islands is 11.4 cm yr^{-1} ($A = 48^\circ 3/4$), and the absolute motion of the Nazca Plate at the same point is 7.1 cm yr^{-1} ($A = 71^\circ 3/4$). According to Johnson and Lowrie¹², the Galapagos hot spot is generating the Cocos and Carnegie Ridges. Our azimuths coincide exactly with the Cocos and the Malpelo Ridges. This is consistent with the idea that the Malpelo Ridge was formed in former times by the Galapagos hot spot on the Nazca Plate, to become extinct when the boundary between both plates, migrating northwards, crossed the ridge. The Carnegie Ridge would be a distinct feature, probably an extinct ridge like the Alpha Cordillera of the Arctic Basin.

The Walvis Ridge is composite, and does not present a clearcut migration of volcanism¹³. Nevertheless, my theory provides a correct azimuth for its southern part (55° ; $u_z = 4.4 \text{ cm yr}^{-1}$).

Lastly, Duncan *et al.*¹⁴ have discussed the migration of volcanism in central Europe. Using my theory the absolute motion at its western extremity (the Eifel), is 2.4 cm yr^{-1} ($A = 65^\circ$). This velocity is exactly the same as that given by the migration of volcanism, but the direction is consistent with that at the western tip of the volcanic range only. But the central European volcanics do not lie on a straight line, and the opening of the Atlantic may have seriously changed the plate motion in the past, and so the fit is satisfactory.

Thus, for five plates out of eight, in the instances in which, a check is possible, the absolute movement deduced from our theorem is consistent with that deduced from the migration of volcanism. This cannot be mere coincidence, and strengthens the idea that our assumptions are sound: there are no lateral variations in the viscosity (except in a few anomalous areas), and the lower mantle is rigid enough to act as a frame of reference for plate motions. This means that in the future, the determination of absolute velocities could have two important uses. First, they can be used to distinguish recently active volcanic chains which are created by hot spots, in the lower mantle, from other volcanic chains. Second, they could be used to calculate movements in the asthenosphere near sinking slabs and marginal basins, taking into account the absolute velocity of the trench relative to the rigid lower mantle. Such a calculation has never been done so far.

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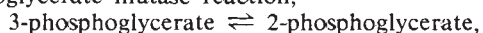
Structure of yeast phosphoglycerate mutase

J. W. Campbell, H. C. Watson & Gail I. Hodgson

Molecular Enzymology Laboratory, Department of Biochemistry, University of Bristol, Bristol BS8 1TD, UK

A 3.5 Å resolution electron density map of yeast phosphoglycerate mutase has been calculated which shows that much of the tertiary structure of this enzyme resembles that found in a number of nucleotide binding enzymes although the mutase itself has no known nucleotide binding requirement.

THE extension of the three-dimensional study of the structure of yeast phosphoglycerate mutase (PGM) by X-ray diffraction to 3.5 Å resolution gives us the first opportunity to report some of the structural details of a 'mutase' enzyme. Rather unexpectedly we find that the most striking structural feature of the enzyme resembles that found in certain enzymes which bind nucleotides¹⁻⁷. The phosphoglycerate mutase reaction,



uses the cofactor 2,3-diphosphoglycerate and has no apparent nucleotide requirement. The results of the present study could therefore provide an important clue in the puzzle of enzyme evolution or alternatively strike a note of caution against over interpretation of results where structural similarities are involved.

Structure determination

The reflection data were measured, in four shells of increasing resolution, on a computer-controlled four-circle diffractometer. The potassium mercuriiodide derivative used in our earlier studies⁸ has now been replaced by a potassium chloroplatinite (5 mM) complex. This new derivative showed better isomorphism than the mercuriiodide derivative though in two experiments it was observed that, after a crystal had been exposed to X-radiation for about 60 h, there seemed to be a rapid and substantial change in the cell parameters. The relevant parameters for the new

derivative are included in Table 1 which summarises the 3.5 Å resolution refinement results. Anomalous dispersion data were measured for both the $\text{K}_3\text{UO}_2\text{F}_5$ and K_2PtCl_4 derivatives. Since our earlier paper we have redefined the origin of the unit cell to coincide with the molecular centre of the tetramer. Independent phase refinement was carried out for each of the four resolution shells of reflections, cycles of maximum probability phase refinement being alternated with the least squares refinement of the heavy atom parameters⁹. The four sets of phased reflections were scaled together using reflections, measured from a single crystal, covering the complete resolution range to 3.5 Å. An electron density map was calculated using centroid phases for nearly 8,000 reflections (average figure of merit 0.80) and plotted on perspex sheets, stacked perpendicular to the crystallographic two-fold axis, on a scale of 0.5 cm per Å.

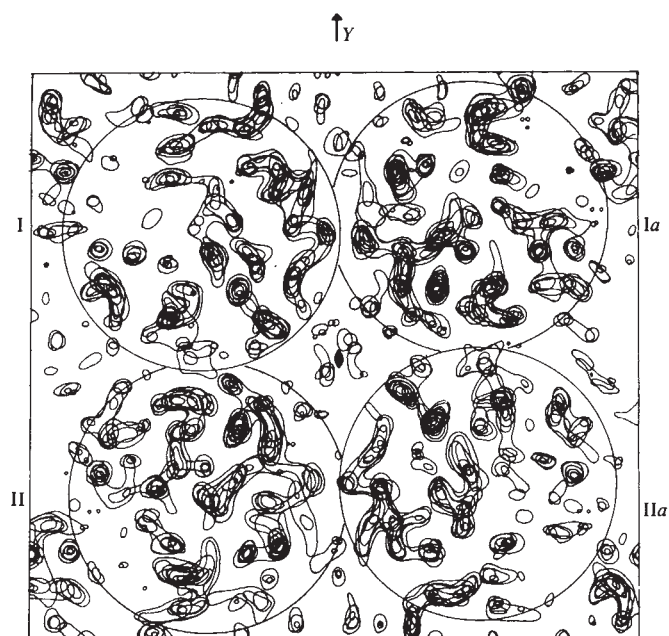


Fig. 1 Part of the yeast phosphoglycerate mutase 3.5 Å electron density map sectioned normal to one of the local molecular two-fold axes. The other local two-fold axis relates subunits I and II, and Ia and IIa respectively. The direction of the crystallographic two-fold axis, Y, is also indicated.

Table 1 Summary of phase refinement

Shell number	1	2	3	4
Resolution range	6.0–15.0 Å	4.4–6.0 Å	3.8–4.4 Å	3.4–3.8 Å
No. of reflections	1,438	2,242	2,134	2,039
R fac.	0.50	—	—	—
tors	0.41	0.47	0.52	0.49
E/f	0.45	0.45	0.58	0.65
Average figure of merit	0.82	0.83	0.78	0.77
Averaged heavy atom parameters				
Derivative	Site number	Relative occupancy	x	y
$\text{K}_3\text{UO}_2\text{F}_5$	I	1.00	0.151	–0.220
	II	0.94	0.164	0.220
	I	0.57	0.020	–0.120
	II	0.75	0.020	0.130
K_2HgI_4	III	1.36	0.183	–0.001
	IV	0.76	0.310	0.771
	I	0.73	0.060	–0.085
	II	0.79	0.237	0.088
K_2PtCl_4	III	0.15	0.429	0.795
	IV	0.15	0.142	0.704
	I	0.15	0.429	0.795
	II	0.15	0.142	0.704

Space group C2; $a = 96.6 \text{ Å}$, $b = 86.0 \text{ Å}$, $c = 81.8 \text{ Å}$, $\beta = 120.6^\circ$. Local 2 fold axes at $y = 0.0$, E r.m.s. lack of closure error, f r.m.s. heavy atom structure factor.

Electron density map

Detailed visual inspection of the averaged and normal electron density maps showed no significant differences between the two subunits within one asymmetric unit, thus confirming the 222 symmetry of the PGM tetramer indicated in the previous structural work on the enzyme^{8,10}. Part of the electron density map showing the local two-fold symmetry is reproduced in Fig. 1. The fact that the two non-crystallographically related but chemically equivalent¹¹ subunits have apparently identical conformations gives us confidence in the accuracy of the structure determination and also indicates that the conformation of the subunit is stable and not unduly influenced by intermolecular forces within the crystal.

The subunits were named as shown in Fig. 1 with I and II being a pair of subunits in an asymmetric unit and with

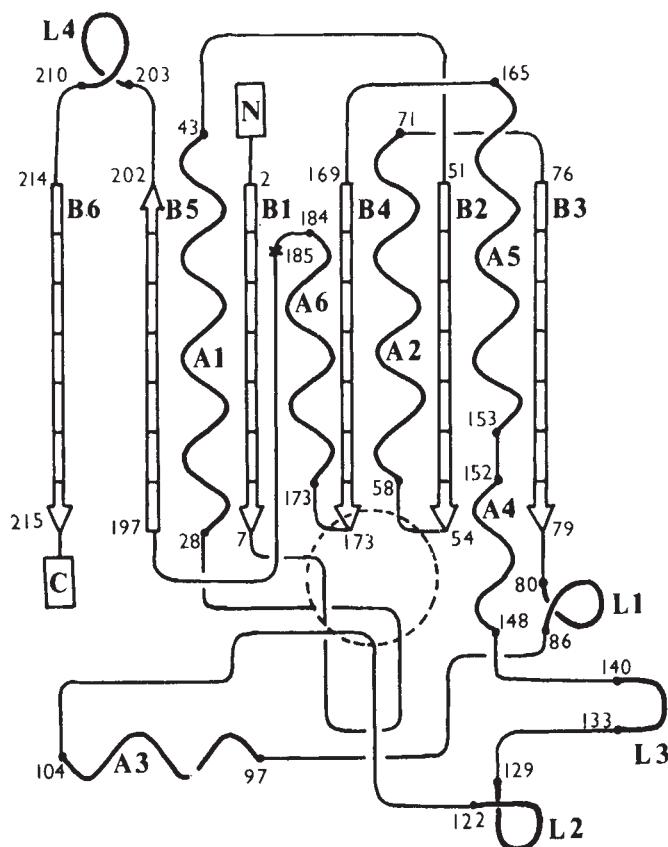


Fig. 2 Schematic representation of the structure of yeast phosphoglycerate mutase showing its regions of secondary structure. These regions are numbered from the N-terminal end of the polypeptide chain with A1–A6 being α -helices, B1–B6 being strands of the β -pleated sheet and L1–L4 being surface loops. The extent of each element of secondary structure can be calculated from the residue numbers marked on the figure. The area enclosed by the broken circle indicates the proposed region for the active site of the enzyme.

Ia and IIa being related to them by the crystallographic two-fold axis. A reasonable course was followed for the polypeptide chain throughout subunit II. In the few places where the interpretation of the map required that the polypeptide chain had to bridge a small gap in the electron density, it was usually found that the density in the map associated with the corresponding point in subunit I was continuous. The interpretation of the electron density map for subunit II fitted equally well the density associated with subunit I apart from small regions near the chain termini. These are at the surface of the molecule where, in general, the electron density is slightly less well defined than for the interior of the molecule. There is no suggestion, however, that the conformations of the subunits are fundamentally different.

The positions of many side chains were clearly visible in the electron density map and this information was used to determine the probable chain direction in all major helical regions. In each case the direction was consistent with the interpretation of the map based merely on the continuity of the electron density. Throughout the map, coloured markers were placed on the probable α -carbon atom positions indicating a total of 220 residues, some 30 less than has been predicted for this enzyme subunit based on molecular weight and amino acid composition data¹². Although a number of forms of the enzyme have been reported^{13,14} in which up to nine residues have been removed from the subunit by autolysis, the present crystallographic result would suggest that the molecular weight may be

slightly lower than 110,700.

In yeast PGM there are six strands forming a β -pleated sheet. The first four strands encountered when tracing the chain from the amino terminus are all parallel and the remaining two strands form an antiparallel pair (Fig. 2). There are five α -helical regions flanking the pleated sheet region (Fig. 3a, b) and, in addition, a further α -helical region making six in all. These α -helices together with helical segments in the regions 11–15 and 108–113 (Fig. 2) account for some 80 residues which, when taken with the 27 or so residues contained in the β -pleated sheet region, indicate that almost half the residues in the molecule are involved in some form of major secondary structure.

The contact between the mutase subunits related by the crystallographic two-fold axis is not very extensive involving mostly the loop L3 (for nomenclature see Fig. 2) from each subunit and the helix A5. The contact between the subunits I and II is much more extensive and involves the side chains of helix A2, the main chain leading into helix A2 and the main chain coming out from this helix which also forms one edge of the β -pleated sheet. The quaternary structure of yeast PGM is illustrated in Fig. 4.

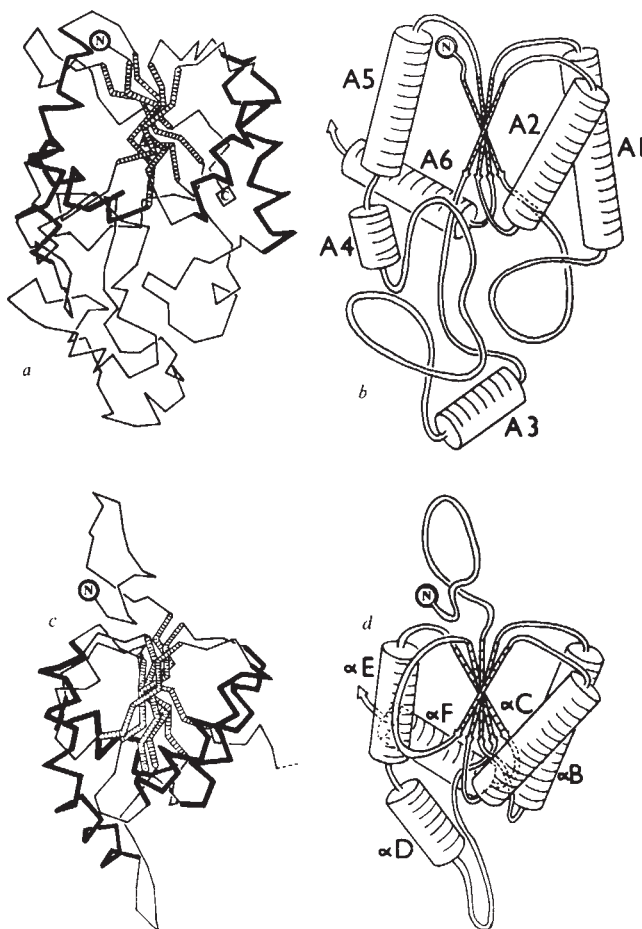


Fig. 3 a, Complete mutase subunit in which strands of the β -pleated sheet have been emphasised as thick shaded lines and in which the α -helices on either side of the β -pleated sheet have been drawn with thick black lines. b, First 185 residues of the mutase subunit drawn in a simplified manner to illustrate the chain folding within this region of the subunit. The helices are labelled using the nomenclature defined in Fig. 2. c, First 165 residues of the lactate dehydrogenase structure drawn for comparison with the mutase subunit in a. The coordinates used in preparing this drawing were from Adams *et al.*¹⁷. d, First 150 residues of LDH drawn diagrammatically for comparison with the part of PGM illustrated in b. The helices are labelled using the nomenclature of Rossmann *et al.*^{1,2}.

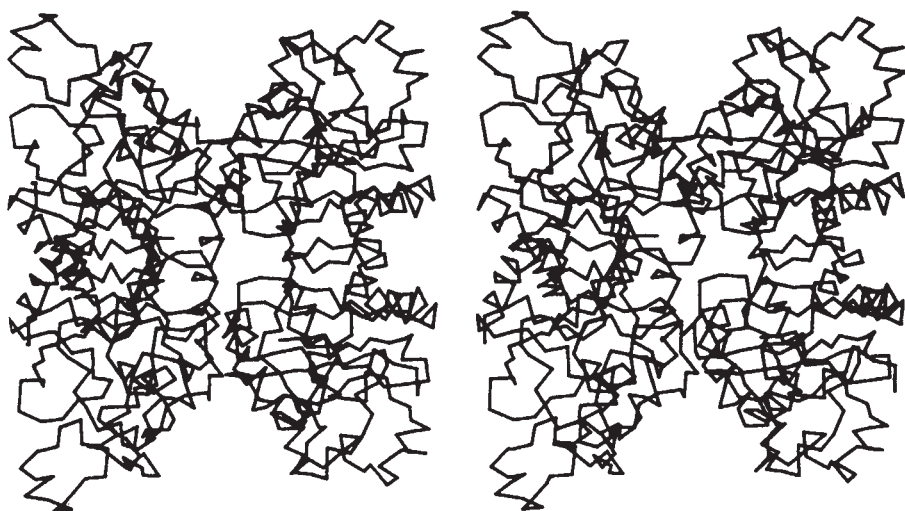


Fig. 4 A stereographic drawing of the yeast phosphoglycerate mutase tetramer. All four subunits are drawn as being identical. The view is chosen to give as clear a picture as possible of the inter-subunit contacts.

Mutase structure

In the absence of primary sequence data and conclusive substrate binding work the most interesting result of the current study is the striking resemblance that part of the mutase structure seems to bear to a structural feature found in the dehydrogenases¹⁻⁵ and the kinases^{6,7} in which a region of β -pleated sheet flanked by α -helices seems to have a common folding pattern. Detailed comparison (Fig. 3) of part of the mutase structure (the first 185 residues involving the first four parallel strands of the β -sheet and helices A1-A6) and part of LDH (the first 150 residues involving the first five parallel strands of the β -sheet and helices α B- α F) suggests that the similarity is more than superficial as evidenced by the fact that the helices A1, A2, A4, A5 and A6 of PGM are connected in the same order along the polypeptide chain as the corresponding helices in LDH. There are however significant differences, namely that, in the region described, PGM has one less strand in the β -sheet and the order of the strands in the sheet is not the same. We cannot see how the present electron density map can be reinterpreted to give the exact LDH folding but it is nevertheless quite possible that the mutase folding, as reported here, has evolved from a protein with LDH folding. The change would have resulted from the insertion of a loop of about 35 residues in a position near strand β D causing this strand to be disrupted from the β -sheet with the consequent rearrangement of two of the remaining strands as shown in Fig. 5.

It is also interesting to note that the substrate binding region tentatively proposed for the mutase in our earlier

paper⁸ is in a closely similar position relative to the α - β structure as that found for the nucleotide substrates of both the kinases and dehydrogenases¹⁻⁷.

Evolutionary significance

It has been suggested^{15,16} that the α - β structure, observed first in the dehydrogenases and more recently in the kinases, is a common feature of these enzymes relating their nucleotide binding function. The presence of a similar feature in the mutase, where no nucleotide binding is required, suggests that this particular structural feature is an easily formed stable conformation which could serve as a suitable core for building intracellular enzymes but may also reflect an ancestral gene from which enzymes in one or a group of related biochemical pathways have evolved.

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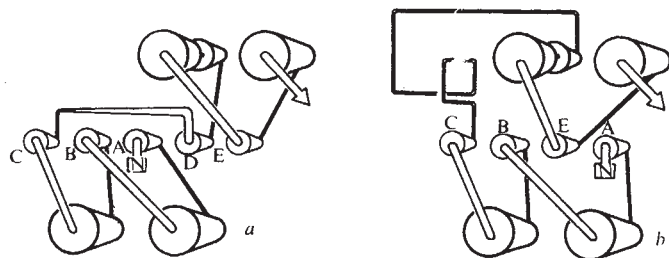


Fig. 5 Diagrammatic representations of the first 150 residues of LDH (a) and of the first 185 residues of PGM (b) showing that the two structures have the same linear connectivity even though the order of strands in their β -pleated sheets is slightly different. Strands of the β -pleated sheet are lettered according to the LDH numbering scheme^{1,2} although the prefix β has been omitted. The flanking helices (see text) are shown above and below their respective pleated sheets.

Effects of succinyl-con A on the growth of normal and transformed cells

Ian S. Trowbridge & David A. Hilborn

The Armand Hammer Center for Cancer Biology, The Salk Institute, P.O. Box 1809, San Diego, California 92112

Covering con A binding sites on virally transformed mouse fibroblasts with succinyl con A has no significant effect on their growth pattern.

CULTURED mouse 3T3 fibroblasts and their viral transformants, SV40-transformed 3T3 (SV3T3) and polyoma-transformed 3T3 (Py3T3) cells, serve as models to demonstrate the differences in growth control between normal and malignant cells^{1,2}. Under normal culture conditions the growth of 3T3 cells and SV3T3 cells is controlled by serum growth factors^{3,4}. As these factors become exhausted, 3T3 cells stop growing and arrest in the G₁ phase of the cell cycle without loss of viability⁵. Under identical conditions SV3T3 cells grow to a much higher cell density than 3T3 cells⁶ as a result of a reduced serum requirement⁷. SV3T3 cells do not arrest in the G₁ phase as serum factors are depleted. Instead they continue to grow, moving slowly through all stages of the cell cycle before they eventually die^{8,9}.

Table 1 Minimum concentration of lectin required for agglutination of cells

Cells	Lectin concentrations ($\mu\text{g ml}^{-1}$)	
	Succinyl con A	Con A
SV3T3	250	10
3T3	>1,000	50

Cells were removed from the dishes by successive washes with 0.15 M NaCl–0.01 M sodium phosphate pH 7.2 (PBS) and with PBS containing 5×10^{-8} M EDTA, followed by a 10 min incubation at 37° C in PBS containing 5×10^{-5} M EDTA²⁶. The cell suspension was washed once with PBS and the cells resuspended at 2×10^6 cells ml⁻¹. Agglutination assays were performed in Linbro 16 mm multi-well tissue culture plates²⁷. Cells (0.2 ml) were mixed with lectin (0.2 ml) and the trays placed on a rotary table²⁸ (1–2 Hz) for 20 min at room temperature. Agglutination was estimated using an inverted light microscope.

It is possible that changes in the cell membrane are responsible for malignant growth^{10–12}. Recently it was reported that Py3T3 cells grown in the presence of trypsinised concanavalin A (con A) revert to normal growth¹³. Native con A is a plant lectin which binds terminal α -D-glucosyl and α -D-mannosyl sugar residues^{14,15}, and it was suggested that the binding of trypsinised con A to glycoproteins on the surfaces of Py3T3 cells caused the reversion to normal growth.

Although originally described as monovalent, subsequent characterisation has shown that protease-treated con A is a complex mixture which includes unmodified protein and totally inactive digest products¹⁶. The complexity of trypsinised con A makes analysis of its biological effects difficult. Extensive succinylation of con A converts the native tetrameric molecule to a dimer with the same binding affinity as the native molecule for methyl α -D-glucoside¹⁷. Succinyl con A is less toxic than the native lectin and does not readily agglutinate cells^{17,18}. Because succinyl con A has a low toxicity but has a similar binding specificity to that of the native lectin, we tested its effects on the growth of normal and transformed cells.

Agglutination and lectin binding

Agglutination studies and lectin binding assays were performed with succinyl con A to find conditions under which the con A binding sites on the cell surface of SV3T3 cells could be covered without inducing cell agglutination. Twice crystallised con A (Miles–Yeda, Kankakee, Illinois) was used without further purification; succinyl con A was prepared and characterised as described¹⁷. Cells from confluent cultures of 3T3 and SV3T3 cells were used in the agglutination assays and the specificity of the lectin-induced agglutination was demonstrated by the inhibition of agglutination by 100 mM methyl α -D-mannoside. Though con A agglutinated SV3T3 cells at a concentration of about 10 $\mu\text{g ml}^{-1}$, the concentration of succinyl con A required to give detectable agglutination was about 250 $\mu\text{g ml}^{-1}$ (Table 1). Succinyl con A at 1 mg ml⁻¹ did not agglutinate 3T3 cells.

The binding of ¹⁴C-succinyl con A and ¹²⁵I-con A to almost confluent 3T3 and SV3T3 cells attached to plastic tissue culture wells was measured in PBS at 4° C. Low temperature was chosen to minimise endocytosis of lectin molecules bound to the cell surface¹⁹. Since the binding of succinyl con A and con A was complete after 30–40 min, cells were incubated with various concentrations of lectin for 50 min. Although succinyl con A did not agglutinate cells as efficiently as the native lectin, succinyl con A bound almost as well as con A to the surface of SV3T3 cells (Fig. 1), and in other experiments bound to the surface of 3T3 cells as well as the native lectin.

Binding experiments were also carried out in PBS at 37° C in the presence of 10% calf serum to measure binding under the conditions of the growth experiments described later. Under these conditions succinyl con A bound more efficiently than the native lectin although the maximal number of molecules of either lectin bound under these conditions was slightly reduced: at a concentration of 100 $\mu\text{g ml}^{-1}$ succinyl con A, 4.2×10^6 molecules per cell were bound and more than 90% of the available binding sites were saturated.

Competition binding studies were carried out to confirm that succinyl con A bound to the same receptor sites as the native lectin. Succinyl con A could inhibit the binding of at least 60% of the ¹²⁵I-con A molecules which bound to SV3T3 cells (Fig. 2). Succinyl con A did not, however, inhibit binding as efficiently as the native lectin. Taken together the results of the agglutination studies and binding experiments suggest that succinyl con A can be used to cover con A binding sites on SV3T3 and 3T3 cells without inducing cell agglutination.

Growth studies

The effects of succinyl con A on the growth of SV3T3, Py3T3 and 3T3 cells have been observed over a wide range of succinyl con A concentrations and under a variety of different growth conditions. For most experiments the effects of succinyl con A at a concentration of 100 $\mu\text{g ml}^{-1}$ were tested since the binding studies showed at this concentration more than 90% of the maximal number of succinyl con A molecules were bound.

3T3 cells and SV3T3 cells were obtained from Dr R. Dulbecco, and Py3T3 cells from Dr W. Eckhart. Cells were maintained in Dulbecco's and Vogt's modification of Eagle's medium supplemented with 10% calf serum, 250 units ml^{-1} penicillin and 50 $\mu\text{g ml}^{-1}$ streptomycin at 37°C in a water-saturated atmosphere at 10% CO_2 and 90% air. No mycoplasma were detected by autoradiography. Cells ($3\text{--}5 \times 10^4$) were seeded in 30 mm Nunc plastic tissue culture dishes in 1 ml of culture medium containing 10% calf serum. The next day (day 0) the medium was replaced with fresh medium (2 ml) containing 10% calf serum and the required concentration of succinyl con A. Cell number in duplicate cultures was determined daily by removing the cells from the dishes with 0.05% trypsin and counting the cells in a Coulter counter. Similar results were obtained if replicate cell counts were made on cells removed from the dish with trypsin containing

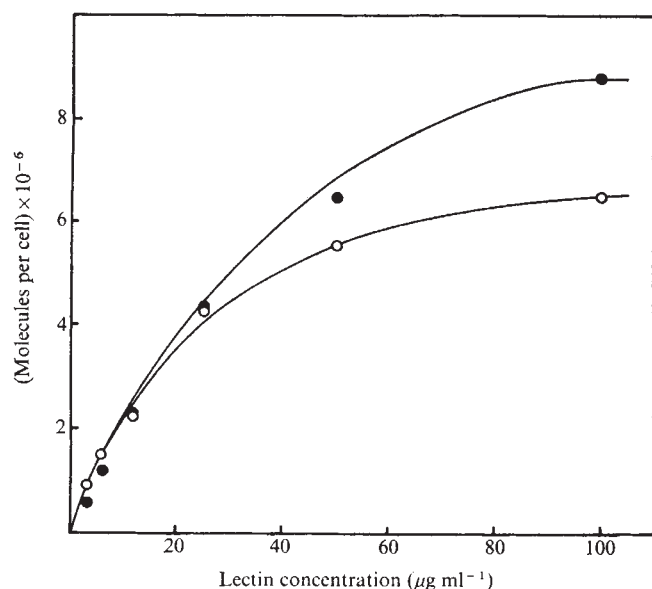


Fig. 1 Binding of ^{14}C -succinyl con A ($\circ$), and ^{125}I -con A ($\bullet$) to SV3T3 cells. ^{125}I -con A (specific activity 7×10^5 c.p.m. μg^{-1}) was prepared by the method of McConahey and Dixon²⁹, and purified by affinity chromatography on Sephadex. ^{14}C -succinyl con A (specific activity 2.5 or 6×10^3 c.p.m. μg^{-1}) was prepared using ^{14}C -succinic anhydride (New England Nuclear). All lectin solutions were filtered through $0.45 \mu\text{m}$ Millipore filters just before use. Cells were grown to 1×10^5 – 2×10^5 cells per well in 16 mm plastic multi-well tissue culture plates (Linbro, Los Angeles). Cell monolayers were washed twice with PBS (1 ml) and incubated with lectin in 0.3 ml PBS for 50 min at 4°C . After incubation cells were washed five times with PBS (1 ml), removed from the wells by overnight incubation with 10% Triton X-100, and the radioactivity bound to the cells determined. Nonspecific binding to the cells was determined as the residual binding in the presence of 100 mM methyl α -D-mannoside. Nonspecific binding of ^{125}I -con A was less than 10% of the total radioactivity bound in the absence of methyl α -D-mannoside. Nonspecific binding of ^{14}C -succinyl con A was 10–20% of the total radioactivity bound. The results reported are corrected for nonspecific binding of the lectins to the cells. Other control experiments showed that in the absence of cells ^{125}I -con A and ^{14}C -succinyl con A bound to wells which had contained only culture medium at 37°C for 24 h. The bound lectin, which was directly proportional to the concentration of free lectin, was reduced by 100 mM methyl α -D-mannoside. Because it is difficult to estimate the contribution of the lectin binding to the plastic (or serum glycoproteins adsorbed to the plastic) in the presence of cells, we have not made a correction for nonspecific binding to the wells. The maximum contribution of lectin bound to the plastic was less than 10% of the total radioactivity measured for the binding of ^{125}I -con A and between 10–30% of the total radioactivity measured for the binding of ^{14}C -succinyl con A.

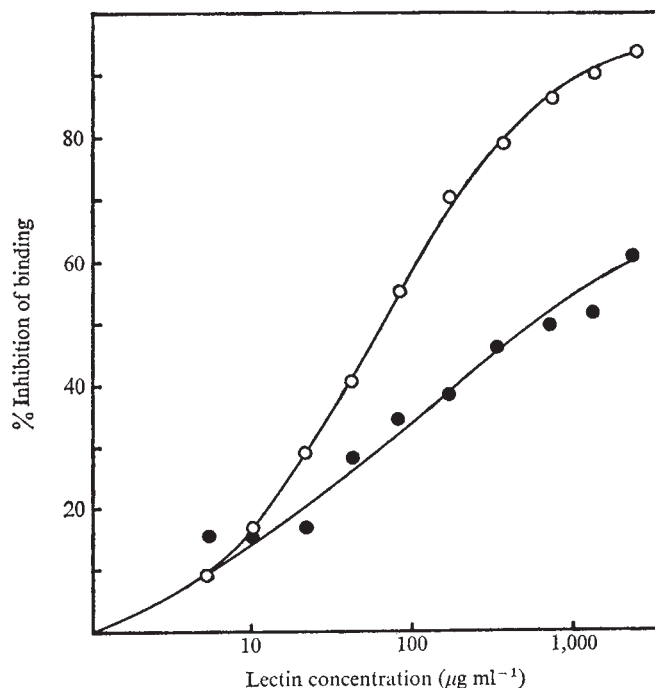


Fig. 2 Inhibition of the binding of ^{125}I -con A ($20 \mu\text{g ml}^{-1}$) to SV3T3 cells by succinyl con A ($\bullet$), and the native lectin ($\circ$). Competition binding studies were carried out as described for direct binding studies. ^{125}I -con A and competing lectin were added to the cells as a mixture in 0.3 ml PBS. No corrections for nonspecific binding were made.

50 mM methyl α -D-glucoside to prevent possible lectin-induced agglutination of the trypsinised cells.

The growth rate and maximum cell density of SV3T3 cells and 3T3 cells was slightly reduced by succinyl con A (Fig. 3). But SV3T3 cells treated with succinyl con A at a concentration of $100 \mu\text{g ml}^{-1}$ still grew to a high cell density before growth stopped and the cells began to die. 3T3 cells treated with succinyl con A stopped growing at a low density and maintained viability. The effects of

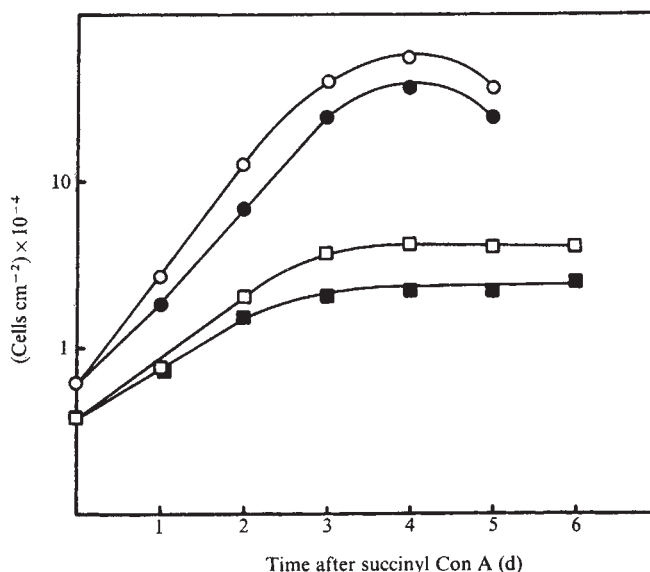


Fig. 3 Effect of succinyl con A ($100 \mu\text{g ml}^{-1}$) on the growth of SV3T3 and 3T3 cells. ($\circ$) SV3T3; ($\bullet$) SV3T3 + $100 \mu\text{g ml}^{-1}$ succinyl con A; ($\square$) 3T3; ($\blacksquare$) 3T3 + $100 \mu\text{g ml}^{-1}$ succinyl con A.

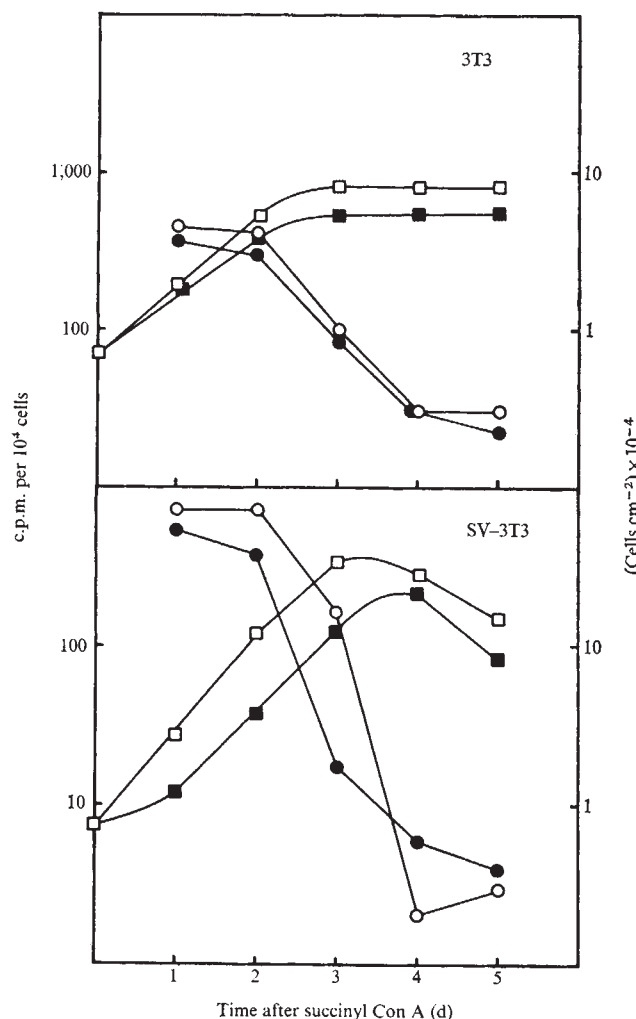


Fig. 4 Effect of succinyl con A ($100 \mu\text{g ml}^{-1}$) on DNA synthesis in SV3T3 and 3T3 cells. (○) ^3H -thymidine incorporation (c.p.m. per 10^4 cells) of cells grown without succinyl con A; (●) ^3H -thymidine incorporation of cells grown with $100 \mu\text{g ml}^{-1}$ succinyl con A; (□) cell density in the absence of succinyl con A; (■) cell density in the presence of succinyl con A. Cells were grown in 1 ml of culture medium which was not changed during the experiment.

succinyl con A on the growth of both cells could be abolished by 50 mM methyl α -D-mannoside in the culture medium demonstrating that specific binding of succinyl con A to the cells was necessary to produce the effects on growth. Succinyl con A had no detectable effect on either the morphology of individual cells or cell cultures.

In the experiment shown in Fig. 3 the culture medium was unchanged throughout the experiment. In other experiments with SV3T3, Py3T3 and 3T3 cells, medium was either partially or completely replaced daily with fresh medium containing the same concentration of succinyl con A. The results of these experiments were the same as those shown in Fig. 3, except that, as expected, all cells grew to higher cell densities. It is therefore unlikely that the reason succinyl con A has little effect on the growth of SV3T3 cells or 3T3 cells is because it is being rapidly degraded by proteolytic enzymes either of cellular origin or present in the serum. In experiments in which culture medium was replaced daily, SV3T3 cells, and to a lesser extent Py3T3 cells, treated with succinyl con A showed a variable tendency to detach from the dish immediately after the culture medium was changed so that experiments with these cells were sometimes difficult to follow for the desired time.

In growth experiments in which the succinyl con A dose was varied, $200 \mu\text{g ml}^{-1}$ succinyl con A (the highest dose tested) inhibited growth of 3T3 and SV3T3 cells only slightly more than $100 \mu\text{g ml}^{-1}$ succinyl con A. When the degree of growth inhibition was plotted as a function of the succinyl con A concentration a saturation curve was obtained which closely resembled the succinyl con A binding curve (Fig. 1). This provides further evidence that the inhibition of growth results from the binding of succinyl con A which occurs to an extent predicted by the binding assays. In contrast to the effects of trypsinised con A on the growth of transformed and normal cells previously reported¹³, succinyl con A inhibited the growth of normal and transformed cells to the same extent at each concentration tested.

Cell cycle analysis

In some growth experiments the rate of DNA synthesis in cell cultures was estimated in replicate cultures by measuring the incorporation of ^3H -thymidine ($2.5 \mu\text{M}$; 1.0 Ci mmol^{-1}) into trichloroacetic acid-insoluble material during a 2 h period (Fig. 4). Daily determinations were made in triplicate. The rate of cellular DNA synthesis remained relatively constant during the first two days of culture when the cells were growing exponentially. As growth slowed there was a large decline in DNA synthesis in cultures of 3T3 cells and SV3T3 cells whether or not the cells were grown in the presence of succinyl con A.

Although DNA synthesis declined in cultures of both normal and transformed cells, microfluorometric cell cycle analysis^{20,21} revealed a profound difference in their growth pattern (Fig. 5). As 3T3 cells reached their saturation density and ^3H -thymidine incorporation dropped, there was a progressive reduction in the proportion of cells in S phase and an accompanying increase in the proportion of cells in G_1 phase. In contrast, no change in the distribution of SV3T3 cells throughout the cell cycle could be detected as their growth slowed. Succinyl con A had no effect on the cell cycle distribution of either cell type at any stage of growth.

The results from microfluorometric analysis and ^3H -thymidine incorporation are consistent with current knowledge of the growth patterns of SV3T3 cells and 3T3 cells. 3T3 cells stopped growing as serum factors are depleted and arrest in G_1 phase^{5,7}, whereas SV3T3 cells continue to grow and eventually die. The dramatic drop in ^3H -thymidine incorporation as SV3T3 cells reach saturation density represents an extreme example of the lengthening of all stages of the cell cycle as nutrients begin to limit their growth^{8,9}.

Our studies provide no evidence that succinyl con A, which binds to con A binding sites, restores the growth pattern of transformed cells to normal. These results are in conflict with the hypothesis that covering of the con A binding sites on virally transformed cells restores normal growth^{13,22,23}. This hypothesis was based upon the effects of trypsinised con A on the growth of Py3T3 cells. Although we used SV3T3 cells in most of our experiments, in other experiments Py3T3 cells treated with succinyl con A also grew to a high cell density. Thus the inability of succinyl con A to restore normal growth seems to be a general phenomenon.

Several explanations could account for the different effects of succinyl con A and trypsinised con A on cell growth. Because of the heterogeneity of the con A binding sites and the failure to completely inhibit ^{125}I -con A binding with succinyl con A, we cannot formally exclude the possibility that succinyl con A and trypsinised con A bind to different fractions of the total receptor population. It should be stressed, however, that this is an unlikely explanation because although no quantitative studies on

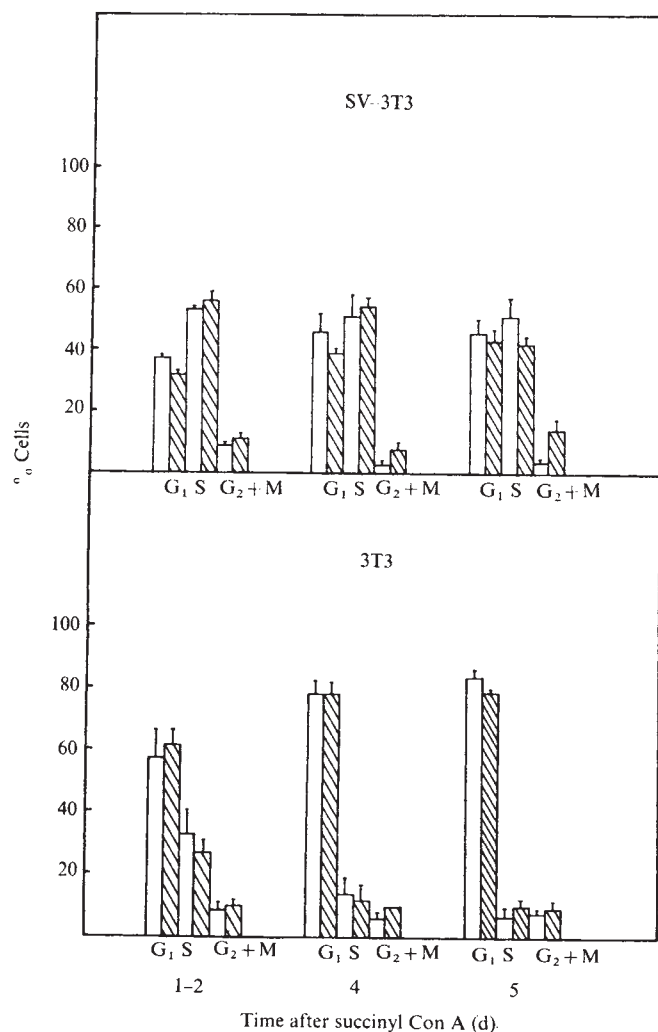


Fig. 5 Cell cycle analysis of SV3T3 and 3T3 cells grown with or without succinyl con A ($100 \mu\text{g ml}^{-1}$). Cells were prepared for flow microfluorometry as described^{20,21}. Cells were removed from the dishes, fixed with formalin and stored at 4°C until the end of the experiment. Cell samples were then stained with acroflavine, resuspended in water and analysed for DNA content using a flow microfluorometer equipped with an argon laser ($\lambda 488 \text{ nm}$)²¹. The vertical bars represent the percentage of the cell population in G_1 , S and G_2+M stages of the cell cycle during exponential growth (day 1 or 2) and as the cells reach saturation density (days 4 and 5). Open bars: untreated cells; hatched bars: cells treated with succinyl-con A. The results are the average values from three experiments ± 1 s.e.

the binding of trypsinised con A to the surface of transformed cells have been reported, Burger and Noonan¹³ observed a graded response of cell growth to different doses of trypsinised con A, $10 \mu\text{g ml}^{-1}$ trypsinised con A significantly reducing the saturation density of Py3T3 cells. Binding experiments (Fig. 1 and ref. 24) show that even the native lectin at a concentration of $10 \mu\text{g ml}^{-1}$ saturates less than 25% of the con A binding sites. No differences in the binding of succinyl con A and con A were observed until a lectin concentration of $50 \mu\text{g ml}^{-1}$ when more than 50% of the con A binding sites were occupied.

Alternatively succinyl con A may bind to the same sites as trypsinised con A but after binding behave differently. For example, succinyl con A, in contrast to the native lectin, neither induces clustering of its own receptors nor inhibits clustering of immunoglobulin receptors on the surface of mouse lymphocytes¹⁷. If this should be the case then succinyl con A will be invaluable in identification of the biologically significant events which occur after the binding of trypsinised con A.

A third possibility is that normal growth control is not restored by treating transformed 3T3 cells with trypsinised con A but that trypsinised con A, like the native lectin²⁵, differentially kills transformed cells. In the original experiments of Burger and Noonan¹³ none of the experimental results unambiguously distinguishes between the possible toxicity of trypsinised con A and its ability to induce transformed cells to arrest in G_1 phase and maintain viability. In particular, our experiments indicate that a decline in thymidine incorporation in cultures of transformed cells is insufficient evidence to justify the assumption that the cells are arresting in G_1 phase.

We conclude because of the lack of effect of succinyl con A on the growth pattern of transformed cells that it is unlikely that the covering of con A binding sites on these cells is alone sufficient to restore normal growth.

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letters to nature

Redshift of a galaxy near 4C11.50

NEAR the position of the radio source 4C11.50, Wampler *et al.*¹ have found a pair of QSOs separated by 4.8 arc s, with redshifts of 0.4359 and 1.901, respectively. Hazard *et al.*² have noted the presence of a 19-mag galaxy, 10 arc s west of the brighter, lower redshift QSO (4C11.50a); a plate obtained with the 224-cm telescope at Mauna Kea shows that this 'galaxy' is actually a close group of three galaxies. Several spectrograms have been obtained at 190 and 50 Å mm⁻¹, with the two brighter galaxies aligned along the slit at position angle 33°. There is a continuum break downwards to the blue at about 5,700 Å, and an emission line at 5,344.9 Å; these features can be identified with the long wavelength edge of the H and K lines, and with the [O II] λ3,727 doublet, respectively. Assuming an average wavelength of 3,727.4 Å for the [O II] doublet, the redshift is 0.4340 (not corrected for galactic rotation). The two galaxies on the slit are separated by about 2.5 arc s and are not well resolved on the spectrogram, so it is not certain to which one the redshift refers.

The redshift of 4C11.50a itself, determined from the sharp [O III] λλ4,959, 5,007 lines, is found to be 0.4358, in good agreement with the value of 0.4359 given by Wampler *et al.*¹. The difference in radial velocity between the QSO and the galaxy, in the local rest frame of the QSO, is 376 km s⁻¹. This observation is consistent with a physical association between 4C11.50a and the galaxy, and supports a cosmological interpretation of the redshift of the QSO.

Burbidge³ has pointed out that the sort of galaxy which observers tend to select for this sort of exercise is normally in the magnitude range which will give it a redshift similar to that of the nearby QSO. Although this may be true in a rough sense, there cannot be very much accuracy with this kind of preselection. Even if the dispersion in the magnitude of the galaxies is ignored, these magnitude estimates of faint galaxies from casual inspection of the Palomar Sky Survey prints are unlikely to have accuracies greater than ± 0.5 mag. That introduces an uncertainty of approximately ± 20% in the redshift, or ± 15,000 km s⁻¹ in the relative velocity for redshifts near 0.4. 4C11.50a is the third reported case in which an intrinsically bright (on the cosmological interpretation of the redshift) QSO has a redshift within a few hundred km s⁻¹ of the nearest galaxy visible on the Palomar Sky Survey prints. The other two are PKS2251 + 11 (refs 4 and 5) and 4C37.43 (ref. 6).

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ALAN STOCKTON

*Institute for Astronomy,
University of Hawaii,
Honolulu, Hawaii 96822*

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Search for optical pulsations from Cen X-3

THE optical system recently identified¹ with the X-ray source Cen X-3 is an obvious candidate for high frequency optical studies because it provides the possibility of observing optical effects related to the 4.842 s X-ray pulsations². Observations of this object were made at Cerro Tololo using an S-20 photomultiplier with no filter and making 1-ms integrations on to magnetic tape. The data were analysed by Fourier techniques similar to those used in the HZ Her study³. No clear harmonic activity emerged, and the average upper limits of the optical pulsations for three sets of data were found to be 0.031%, 0.11%, and 0.14% (Table 1).

Table 1 Time series observations of Cen X-3

Date	Telescope (cm)	Starting UT	Phase*	Length (min)	Upper limit (%)
January 24/25, 1974	152	0528	0.691	50	0.031
February 21/22, 1974	91	0121	0.024	400	0.11
February 22/23, 1974	91	0133	0.508	400	0.14

* See ref. 2.

There is, however, a tantalising peak at exactly the Doppler-shifted frequency of pulsation, at the 1.3 σ level. This appeared for an 8 min interval beginning at 0133 ut (phase = 0.508) on the night of February 22/23, 1974 (Table 1). This 'activity', corresponding to a fluctuation of 0.25%, should be regarded sceptically; it is reported here in recognition of the possibility that this object may, like HZ Her, display optical pulsations only rarely^{3,4}.

The detected optical pulsations in Her X-1 are of the same fractional order of magnitude as the observed upper limit for Cen X-3 reported here. The X-ray flux from Her X-1 (refs 5 and 6) is about 2.9 times fainter than that for Cen X-3 (refs 7 and 8). The corresponding optical flux—corrected for reddening taking $A_v = 0.3$ (ref. 9) and 4.3 (W. Krzeminski, personal communication) respectively—is 76 times fainter. Thus, if the pulsed optical emission in these kinds of objects is excited by the absorption of X rays, than significant optical activity at the present limit of detection would require a conversion efficiency about 26 times greater for Cen X-3 than for Her X-1. Although further observational work would increase the chances of detecting sporadic activity, a significant decrease from the present upper limit is not achievable with available instrumentation.

BARRY M. LASKER

*Cerro Tololo Inter-American Observatory,
Casilla 63-D,
La Serena, Chile*

Received May 14, 1974.

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Dynamic evidence on massive coronas of galaxies

A LONGSTANDING unresolved problem in galactic astronomy is the mass discrepancy observed in clusters of galaxies. The virial mass of the cluster per galaxy and the mass-luminosity ratio are considerably larger than the corresponding quantities for individual galaxies. This discrepancy cannot be a result of expansion or be because of the recent origin of clusters: these ideas contradict our present knowledge of the physical evolution and ages of galaxies¹. Therefore it is necessary to adopt an alternative hypothesis: that the clusters of galaxies are stabilised by hidden matter.

The discovery of the emission of X rays from clusters of galaxies can be explained in terms of hot intracluster gas. The mass of this gas, however, is insufficient to stabilise clusters².

Another possible way of removing the mass discrepancy is to suppose that the masses of individual galaxies have been underestimated. This hypothesis is supported by the fact that there is a very slow decrease in the rotational velocity of spiral galaxies, which indicates the pressure of large amounts of matter in their outer regions³. On the other hand, the discovery of large halos in elliptical galaxies^{4,5} indicate that these also contain previously unseen matter.

There have been several estimates of the mass of galaxies, based on the new observational data mentioned^{6,7}. A critical analysis shows, however, that these estimates are very uncertain.

We here attempt to obtain more exact information on the distribution of mass in the outer regions of galaxies.

Consider a body moving in a circular orbit of radius R , with a velocity V_0 , around the centre of the galaxy. If mass distribution is spherical, then

$$M(R) = RV_0^2/G$$

is the mass of the galaxy inside the sphere of radius R , where G is the gravity constant. The same formula can be used for a nonspherical mass distribution, neglecting an error of about 10%.

Spiral galaxies contain neutral hydrogen and HII regions moving in the plane of the galaxy in nearly circular orbits. Observations of these objects permit us to calculate the function $M(R)$ in some galaxies at rather large distances from the centre. We have calculated this for five galaxies of different mass. The results for one galaxy (IC342) are given in Fig. 1.

For all galaxies we have also found the mass distribution $M_s(R)$ of known stellar populations—the bulge, the halo and the disk. We assume that these populations are physically homogeneous, in particular that the mass-luminosity ratio of stars is constant for the whole population. The relevant parameters (luminosity, L ; effective radius R_0 ; mass-luminosity ratio, f ; axial ratio of equidensity ellipsoids, ϵ) for all of the populations, have been determined from combined photometric, spectrophotometric, kinematical and radio observations, using previously described methods^{7,8}.

The resulting mass distribution of known stars, $M_s(R)$, deviates considerably from the mass distribution, $M(R)$, derived from the rotation curve. Rejecting the possibility of large systematic deviations from the circular velocity in the outer regions of galaxies, we conclude that galaxies contain

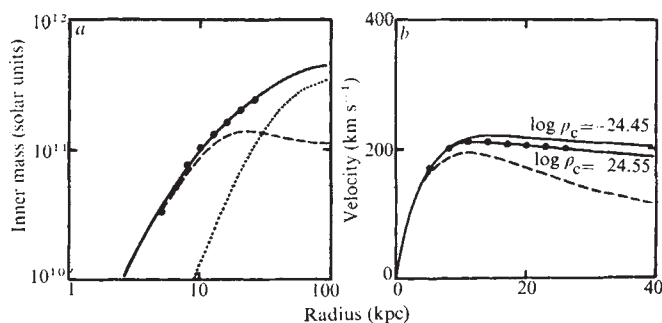


Fig. 1 The distributions of (a) the inner mass, $M(R)$, and (b) the circular velocity, V_c , in the galaxy IC342. Dots, observed values; dashed lines, model functions for known stellar populations; dotted lines, distributions for the corona; solid lines, total distributions. In Fig. 1b two variants of the total velocity distribution are given to demonstrate its dependence on the central density of the corona.

a previously unrecognised, massive population. It is convenient to call this population a 'corona'. We have estimated the parameters of coronas for the five galaxies (Table 1) adopting, for simplicity, an exponential density law

$$\rho(R) = \rho_0 \exp(-R/R_0)$$

with spherical symmetry. (A small axial ratio, ϵ , would result in a large part of the kinetic energy being in the form of rotation, although the results of Ostriker and Peebles do not indicate this⁹.)

The available data permit us to derive the central density of the corona, ρ_0 , with great accuracy. The length of the observed rotation curve, however, is insufficient to allow any determination of the mass and extent of the corona.

To determine total masses and dimensions of galactic coronas, more distant test bodies are needed. For this purpose isolated pairs of galaxies can be used, with secondary galaxies serving as test bodies for determining the mass distribution of primary galaxies¹⁰.

In the case of double galaxies we know the radial component of differential velocity, $v_r = \Delta V_r$. The projection factor, $p = V_c^2/v_r^2$, which depends on the orientation of the velocity vector and on the shape of the orbit, remains unknown, but its expected value, $\langle p \rangle$, can be calculated. Therefore, the mass distribution function can be found only statistically:

$$\langle M(R) \rangle = \langle p \rangle \langle Rv_r^2 \rangle / G,$$

where $\langle Rv_r^2 \rangle$ is the corresponding mean value for a sample of pairs of galaxies.

We have collected data on 105 pairs of galaxies with known types, radial velocities and estimates of magnitudes and dimensions^{11,12}. To prevent the sample from contamination by optical pairs, only those pairs with signs of interaction or with a small differential motion, $\Delta V_r/V_r < 0.23$, have been considered. The distances have been determined from the mean recession velocity, V_r , using the Hubble constant, $50 \text{ km s}^{-1} \text{ Mpc}^{-1}$. For some nearby pairs other distance indicators have been used. The pairs have been divided into five equal groups according to the radius, R . The resulting function $\langle M(R) \rangle$ is shown in Fig. 2.

We have also calculated the average mass distribution of known stellar populations, $M_s(R)$, and have determined the distribution of mass in the corona, $M_c(R)$ (Fig. 2). The resulting central density agrees well with our previous estimates from individual galaxies. The use of distant satellites as test bodies enables us to derive masses and effective radii of coronas with a sufficient accuracy. There are enough pairs in our sample to allow an estimation of the masses of coronas separately for primary elliptical galaxies and also for bright and intermediate spiral galaxies. The estimated parameters are given in Table 2. In the case of primary elliptical galaxies the total masses of coronas may be underestimated.

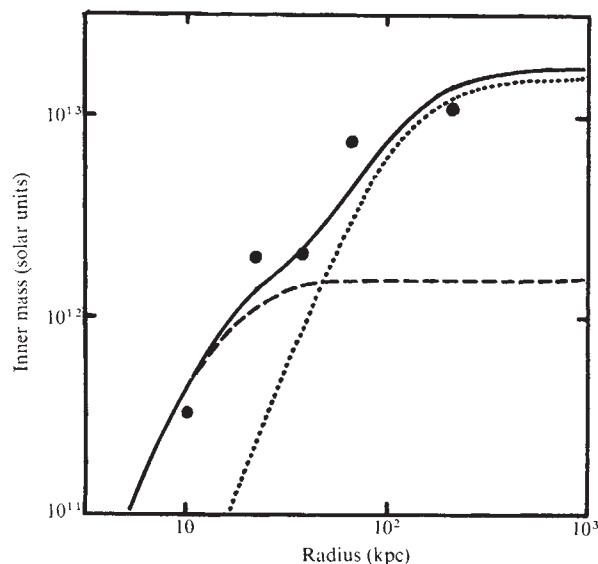


Fig. 2 The distribution of the mean inner mass, $\langle M(R) \rangle$, obtained from 105 pairs of galaxies. Symbols as in Fig. 1.

The mass of galactic coronas exceeds the mass of populations of known stars by one order of magnitude, as do the effective dimensions, by a similar factor. The central density of coronas is surprisingly constant: $\log \rho_c = -24.5 \pm 0.1$ (g cm^{-3}).

The presence of massive coronas in galaxies considerably reduces (if not removes) the virial mass discrepancy in clusters of galaxies. The mass-luminosity ratio rises to $f \approx 100$ for spiral and $f \approx 120$ for elliptical galaxies. With $H = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ this ratio for the Coma cluster is 170 (ref. 1).

According to new estimates the total mass density of matter in galaxies is 20% of the critical cosmological density.

The possible physical nature of galactic coronas will be discussed in a separate article.

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JAAN EINASTO
ANTS KAASIK
ENN SAAR

W. Struve Astrophysical Observatory,
202444 Tõravere, Estonia, USSR

Received April 10, 1974.

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Origin of QSO absorption lines

THE origin of the absorption lines in QSOs, with $z_{\text{abs}} \ll z_{\text{em}}$ has remained a puzzle. It has been suggested that they result from intervening matter lying along the line of sight but not associated with the QSO^{1,2}. It has also been suggested that the absorption lines arise 'locally' from matter expelled from the QSO core³⁻⁶. In particular, radiation pressure has been proposed as the driving mechanism³⁻⁵. It has, however, been shown that the final velocities required by the expelled matter ($v_f \sim c$) for z_{abs} to be $\ll z_{\text{em}}$, cannot be attained by radiation pressure⁷. Alternatively, matter could be accelerated to velocities $v_f \sim c$ by free electron scattering in the outer region of the QSO core⁷.

Any model that attempts to explain the formation of QSO absorption lines must explain the highly multiple z_{abs} spectra that are sometimes seen, and the distribution of the multiplicities. At least 10 z_{abs} per QSO are required to explain the spectra of the multiple z_{abs} QSOs PHL957, Ton1530, PKS0237-23 and 4C05.34 (refs 9-12).

The observed redshifts, z_{abs} ($\ll z_{\text{em}}$), in the local interpretation, correspond to final velocities $v_f \sim 100,000 \text{ km s}^{-1}$ and the absorption line widths correspond to velocity dispersions $\Delta v_f \sim 30 \text{ km s}^{-1}$ (refs 13 and 14). A particularly small Δv_f of $\sim 8 \text{ km s}^{-1}$ has recently been observed¹⁵. The basic problem of the local interpretation is to ascertain how a cloud be accelerated to final velocities $v_f \sim c$ yet have a final velocity dispersion $\Delta v_f \sim 10^{-4}$.

So far the existence of 'plasmoids', consisting of relativistic particles in a magnetic field, is the only mechanism in the local interpretation that has been suggested⁶ to decrease the relatively large possible macroscopic or bulk motions in QSO clouds (velocity dispersions). In the plasmoid mechanism there are several assumptions⁶. First, that an individual plasmoid typically expands adiabatically from $\sim 5 \times 10^{17} \text{ cm}$ to $\sim 10^4 \text{ pc}$; second, that $\sim 10^4$ plasmoids form a single cloud of dimension $\sim 10^4 \text{ pc}$; third, that inhomogeneities in the magnetic field of the single cloud cause density enhancements such that separate regions are created with different z_{abs} ; and fourth, that the different density enhancements have relativistic velocities with respect to one another, thus explaining the large differences in the observed z_{abs} .

As a possible alternative to that plasmoid mechanism, we show here that certain large relative bulk motions in QSO clouds can be dissipated without the presence of strong magnetic fields and without large adiabatic expansions. The essence of the argument is that a cloud with convergent internal velocities can coalesce inelastically because the internal kinetic energy can be dissipated and radiated away.

It is easy to show this when using the known data of QSO absorption clouds. I assume that the cloud, with convergent internal velocities, extends from a distance R_1 to R_2 from the QSO core, and has a velocity profile that decreases with increasing radius ($v(R_1) > v(R_2)$). An ion at R_1 has a relative velocity, $\hat{v}$, with respect to the neutral atoms in the cloud, where $0 < \hat{v} < \hat{v}_m$. The velocity, $\hat{v}_m$, is the maximum dispersion velocity of the cloud ($v(R_1) - v(R_2)$) and is taken to be large ($\hat{v}_m \gg 10^{-4}c$).

The average energy loss for an ion per neutral atom, $\epsilon(\hat{v})$, is approximately inversely proportional to $\hat{E}(\hat{v})$, where $\hat{E}(\hat{v})$ is the relative kinetic energy and $\hat{v}$ is greater than a characteristic velocity, v_0 (ref. 16). Thus, $\hat{E}(\hat{v}) \epsilon(\hat{v}) \simeq \hat{E}(v_0) \epsilon(v_0)$. Because the relative velocity, $\hat{v}$, for an ion at R_1 varies from 0 to $\hat{v}_m$, the range of the ion in the cloud is of the order

$$\bar{\rho} \equiv [\rho E(v_0) \hat{v}_m^3 / v_0^3 \epsilon(v_0) f n(R_1)],$$

where f is the fraction of the atoms of the cloud which are neutral, and $n(R_1)$ is the particle density of the cloud. Assuming that the cloud thickness, $R_2 - R_1$ is comparable to R_1 , then

the condition for the dispersion velocity, $\hat{v}_m$, to be dissipated is

$$\bar{\rho} \ll R_1 \quad (1)$$

The density $n(R_1)$ can be related to the column density of the observed absorption clouds, $N_a \equiv n(R_a)\Delta R_a$, where R_a is the average distance of the absorption clouds from the QSO core, $n(R_a)$ is the particle density of the clouds at R_a , and ΔR_a is the shell thickness in which the observed absorption clouds are found⁷. A high probability exists for finding absorption lines in QSOs with large z . This implies that the observed absorption clouds have a solid angle coverage approaching 4π sr. The mass of the clouds is $M = 4\pi N_a R_a^2$. We assume that $R_1 \ll R_a$. The time to expel the clouds of mass M (now at R_a), through a sphere of radius $R_1 < R_a$, is $< R_a/v_r$. It is also $\geq M/4\pi r^2 v_r n(R_1)$. The density, $n(R_1)$, must therefore obey the relationship $n(R_1) \geq N_a R_a^2$.

The range, $\bar{\rho}$, in equation (1) thus obeys the relationship $\bar{\rho} < \bar{\rho}_m \equiv E(v_0) \hat{v}_m^3 R_1^3 / v_0^3 \epsilon(v_0) f N_a R_a$. Using existing experimental data we can evaluate $\bar{\rho}_m$. $E(v_0)$ for protons in helium as ~ 100 keV. The $\epsilon(v)$ for protons in helium has been measured and found to be $\sim 7.0 \times 10^{-15}$ cm² eV at 100 keV, and $\sim 6.5 \times 10^{-15}$ cm² eV at 40 keV (refs 17-20). Helium atoms in the absorption cloud will be the first atoms to recombine, and so $\epsilon(v_0) \sim 7.0 \times 10^{-15}$ cm² eV can be taken for the evaluation of $\bar{\rho}_m$. Burbidge and Chan²¹ evaluated the column densities of the QSOs PHL938, PKS0237-23 and Ton1530. They found that $N_a \sim 10^{19}$ cm⁻², which we assume to be true. Bahcall and Goldsmith¹⁰ placed a lower limit of 10^3 pc on R_a for the QSO 4C05.34, which we also assume to be true. We then have $\bar{\rho}_m \sim 10^{-4} \hat{v}_m^3 R_1^3 / v_0 f$, where $\hat{v}_m$ and v_0 are in units 10^3 km s⁻¹ and R_1 is in pc.

Reasonable estimates of $\hat{v}_m$, R_1 and f give $\bar{\rho}_m/R_1 \ll 1$ (for example $\hat{v}_m \sim 10^{-2} c$, $R_1 \sim 1$ pc, $f \sim 0.01$). The condition of equation (1) then seems to be satisfied.

It is of interest to estimate the final equilibrium velocity, $\hat{v}_{eq}$, that the particles attain in the cloud. At very low energies, an important factor that must be taken into account is that the particles no longer travel in straight lines. Although traverses in a straight line are a good approximation when $\hat{E}$ is large compared to atomic energies ($\sim I$), this assumption is not valid at very low energies (that is, when $\hat{E} \sim I$). When $\hat{E}$ becomes comparable to I , large transfers of momentum can occur. Rather than scattering primarily in the forward direction, as at high energies, the scattering becomes approximately isotropic for $\hat{E} \sim I$. If ΔE is the average energy loss per collision, then the energy, ΔE , will be small, because most collisions at very low energies are elastic. The ratio of the range, $\rho(\hat{E})$, of a particle, which occurs when large transfers of momentum are important to the range of the particle travelling in a straight line, $\rho_s(\hat{E})$, is $\rho(\hat{E})/\rho_s(\hat{E}) \sim N^{1/2}$, where $N \sim E/\Delta E$. For example, if $\Delta E \sim 0.1$ eV and $E \sim 100$ eV, then the ratio is $\sim 1/30$.

Particles in the cloud will lose energy at an appreciable rate so long as $\hat{E} > \bar{E}_i$, where $\bar{E}_i$ is the average ionisation energy ($\bar{E}_i < I$). The inelastic cross section will rapidly decrease when $\hat{E} < \bar{E}_i$. Thus, an approximately stable equilibrium velocity occurs when $\hat{E}(\hat{v}_{eq}) \sim \bar{E}_i$. Using $\bar{E}_i \sim 13$ eV gives $\hat{v}_{eq} = (2\bar{E}_i/\bar{M}) = 10^{-4} c$, where $\bar{M}$, the average atomic mass, is ~ 1 . It is interesting that this value is approximately equal to the observed dispersion velocity of absorption line clouds, $\Delta v_r (\sim 10^{-4} c)$.

There are thus indications that observed QSO absorption clouds may have their origin in clouds with internal velocities which are convergent, even through the initial velocity dispersion, $\hat{v}_m \gg \Delta v_r \sim 10^{-4} c$, where Δv_r is the observed velocity dispersion. It might be argued, however, that clouds with internal velocities which are divergent and with $\hat{v}_m \gg 10^{-4} c$ are as likely to occur as are convergent clouds. Although this is true it should be noted that if such divergent clouds do exist it is very difficult to observe them. Because of the breadth of the absorption lines, the ratio of the magnitude of a given absorption line from such a cloud, to the magnitude of the line from a con-

vergent cloud, is $\ll 1$. For example, if $\hat{v}_m \sim 10^{-2} c$ the ratio is $< 1\%$.

The gas in a convergent cloud will be very highly ionised. In our discussion we have tried to be conservative and we therefore used $f \simeq 0.01$, that is, the cloud is 99% ionised. The cloud will be heated further because of, for example, adiabatic compression, ion-atom collisions and the formation of shock waves. The mechanism described for the formation of a cloud with a narrow velocity dispersion, requires that the cooling time is shorter than the dynamical scale of time. The question whether the heat can be radiated away before the cloud has time to rebound can only be answered by a detailed analysis of the dynamical equations.

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REUVEN OPHER*

Space Science Division†,
Ames Research Center,
NASA Moffett Field, California

*The author's name has recently been changed from Raymond Fox to Reuven Opher.

†Present address: Physics Department, Technion-Israel Institute of Technology, Haifa, Israel.

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Absorption of gamma rays in intense X-ray sources

It has been proposed that intense X-ray sources could also be candidates for γ -ray sources, but there has been no experimental evidence. This can mean either that no γ rays are produced or that they are absorbed before they leave the source. Under certain assumptions about the geometry of the X-ray source one can show that the γ rays are strongly absorbed as a result of pair production with the X-ray photons. On the other hand, measurements on hard photons above the X-ray band may allow us to derive information on accretion sources by virtue of their absorption mechanism.

The importance of photon-photon absorption in astrophysics was first mentioned by Nikishov¹. He calculated the

absorption of high energy γ rays (10^{12} eV) on thermal photons in intergalactic space. Jelley² considered the same absorption mechanism within possible γ -ray sources. McBreen³ pointed out that γ rays from the Crab nebula should already be absorbed for γ -ray energies > 50 MeV as a result of pair production with the X-ray photon field. The following calculations describe the absorption of γ rays in some intense X-ray sources using recent data.

Two photons of energy E_x , E_γ can produce an electron-positron pair in a collision provided that

$$E_x E_\gamma \geq 2(mc^2)^2 / (1 - \cos\alpha) \quad (1)$$

where m is the electron mass and α is the angle between the directions of motion of the two photons. For a fixed γ energy E_γ the cross section σ is a function of the X-ray energy E_x (ref. 4). The cross section σ rises steeply from a threshold $E_x = E_s$ given by equation (1), it has a maximum value at $E_x = 2E_s$ and falls off as E_x^{-2} for X-ray energies $E_x \gg E_s$.

We describe the absorption of the γ rays by calculating the dimensionless optical depth τ . The γ -ray intensity then varies as $I_\gamma = I_{\gamma 0} \exp(-\tau)$. Detailed calculations of τ assuming an isotropic radiation field are given by Gould and Schreder⁵. For simplicity it can be written

$$\tau = r \int \sigma n_x dE_x \quad (2)$$

taking the cross section σ and n_x , the density of X-ray photons per unit energy, to be constant throughout the source.

Both σ and n_x are functions of the X-ray energy E_x . r is the length along which absorption takes place. Furthermore, we assume that the angle α in equation (1) is 90° . If we approximate σ by a rectangular function with height $\sigma_0 = 1.7 \times 10^{-26}$ cm² and width $2.5E_s$ we get from equation (2)

$$\tau \approx r \cdot \sigma_0 n_x(2E_s) \cdot 2.5E_s \quad (3)$$

where we have chosen n_x at the energy $2E_s$, corresponding to $\sigma_{\max}$.

Equation (3) gives τ as a function of the γ -ray energy E_γ because from (1) we have

$$E_s = 2(mc^2)^2 / E_\gamma \quad (4)$$

To estimate n_x we use observational data⁶⁻⁸. If f is the flux of X-ray photons per unit energy measured on Earth we have approximately

$$n_x = f 4\pi R^2 / 4\pi r^2 c \quad (5)$$

where R is the distance to the X-ray source and r the size of the source which we take to be equal to the length where we have absorption.

To get reasonable values for r we refer to current models of X-ray sources. We calculated the optical depth for three different X-ray sources, Her X-1, Cyg X-1 and the Crab pulsar. For Her X-1 we take $r = 10^5$ cm, the size of the magnetic pole region on the surface of an accreting neutron star⁹. For Cyg X-1 we have $r = 10^6$ cm assuming an accretion disk around a black hole as the X-ray source⁹. In the case of the Crab pulsar we take $r = 3 \times 10^7$ cm an upper limit for the γ -ray source if the observed γ pulse has a halfwidth of about 1 ms.

Figure 1 shows τ as a function of the γ -ray energy E_γ .

It can be seen from Fig. 1 that we cannot expect γ rays above 10 MeV depending on the parameters of the source. On the other hand, the electron-positron pairs produced by the absorption of the γ rays can be partly stopped and they then annihilate. This 511 keV radiation is no longer absorbed by the X-ray photon field and can probably escape depending on the matter density present.

If there are pulsed γ rays from the Crab above 10 MeV as claimed by some authors, this would not contradict the above result. From equation (1) we see, that the threshold energy E_s goes to infinity in the case of beamed radiation ($\alpha = 0$).

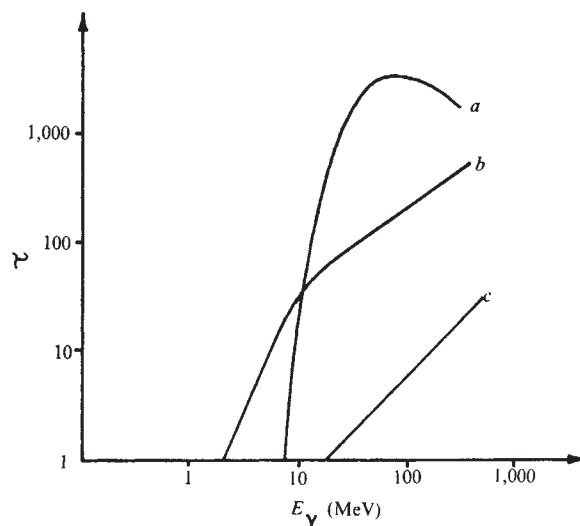


Fig. 1 τ as a function of the γ -ray energy E_γ . a, Her X-1; b, Cyg X-1; c, Crab.

One can speculate whether there exist other sources where the optical depth $\tau(E_\gamma)$ might be less than 1. From equations (3) and (5) we get

$$\tau \alpha L_x / r \quad (6)$$

with $L_x = f 4\pi R^2$. τ can be small for large r and small L_x .

Examples of the first case are Seyfert galaxies. If we take $r = 10^{-2}$ pc, $L_x = 10^{42}$ erg s⁻¹ and compare with the corresponding parameters of Her X-1 ($r = 10^5$ cm, $L_x = 10^{37}$ erg s⁻¹) taking τ from Fig. 1, we obtain a value for τ , in the case of Seyfert galaxies, much less than 1.

An example of the second case could be an optically thin relativistic plasma of say $kT = 10$ MeV becoming thermally unstable¹⁰. Ninety per cent of the energy would then be radiated within the energy range 1 MeV–10 MeV. Certainly, the temperature of such a plasma cannot exceed 20 MeV because of cooling caused by the production of electron-positron pairs¹¹. If there is not much X radiation compared with the γ radiation this also has some influence on the Eddington limit which sets an upper limit for the mass flow in an accreting object. The corresponding maximum luminosity is proportional to σ^{-1} , the cross section in the interaction between the infalling matter and the outgoing radiation field. Thus, we can have higher luminosities for γ -ray sources than for X-ray sources. This factor could be as large as 150 for γ energies above 200 MeV.

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K. HERTERICH

Max-Planck-Institut für extraterrestrische Physik,
8046 Garching b, Munich,
Federal Republic of Germany

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Energy source for comet outbursts

A COMET nucleus is generally recognised as an icy conglomerate, as was originally proposed by Whipple¹. The Orbiting Astronomical Observatory observations of Comet Tago-Sato-Kosaka² and Comet Bennett³ support the current ideas that H₂O is a major component of comets. If comets were indeed formed through an accretion mechanism at distances of many AU from the Sun, what is the nature of the resulting form of water ice?

A number of studies on the deposition of water vapour at low pressures and temperatures indicate that amorphous ice is formed. The reported physical properties include: a density of 2.3 g cm⁻³ (ref. 4), a specific heat 25% greater than that of ordinary hexagonal ice, and a latent heat for the phase transition from amorphous to cubic ice of 24 ± 2 cal g⁻¹ (ref. 5). The transition occurs at a temperature near 140 K (ref. 6). The presence of impurities seems to enhance the growth of clathrate hydrate ices, but both the clathrates and the amorphous water ice can apparently coexist at densities of 1.4–1.7 g cm⁻³ (ref. 4). Clathrate compounds have densities which are typically 0.3–0.5 g cm⁻³ (ref. 7).

Observational evidence indicates that comet outbursts require an internal energy source⁸. If at least the surface of a comet nucleus contains a substantial percentage of amorphous ice, then the phase transition of the amorphous ice to a cubic structure provides a release of energy which may be responsible for the outbursts observed in many comets. In addition, if the density of amorphous ice is indeed about 2 g cm⁻³, then a 'pulverising' mechanism would exist because of the abrupt stresses introduced by the volume change in the solid as the density of the ice changes by a factor of two.

The total energy released during a cometary outburst is of the order of 10²¹ erg with an accompanying mass loss of 10¹² g (ref. 8). The resulting energy requirement of 10⁹ erg g⁻¹ compares favourably with the 10⁹ erg g⁻¹ released during the amorphous-cubic phase change.

Any theory to explain cometary outbursts must consider the spatial distribution of the phenomenon. Figure 1 reports the results of a study in which the positions of the comets are shown at the time of outburst⁹. Although some observational selection effects may be present, a definite clustering is apparent within 2.5 AU from the Sun. Calculations¹⁰ have shown that celestial bodies consisting of water ice with an albedo of 0.6, at a distance of 2.5 AU from the Sun, have expected surface temperatures ranging from 150 K for a rapidly rotating sphere, to 180 K for a non-rotating sphere (see Figs 1 and 2 of ref. 10). The phase transition from amorphous to cubic ice requires a temperature near 140 K. Higher temperatures are necessary if the surface has an insulating layer such as that predicted in the model we shall present here.

Any volume element which undergoes this phase transition increases its temperature by about 45 K. Therefore, the heat released can trigger the surrounding material so that the phase change is, in effect, a self feeding mechanism which propagates to a distance where the local temperature is near 100 K.

The outburst of a comet can therefore be envisaged as occurring in a series of consecutive steps: initiation, propagation, pulverisation, sublimation, ejection, and insulation.

Depending upon the condition of the surface of the comet, the amorphous-cubic ice phase transition can be achieved at different solar distances. If the surface is amorphous ice, an outburst is most probable at about 2.5 AU from the Sun. If the surface is covered with an insulating layer, however, a

closer approach to the Sun is required. On the other hand, if amorphous ice is in heat exchange with material with a temperature which increases faster than that of the ice, then an outburst can be expected at greater distances. If the temperature of the ice is slightly below the transition temperature, thermal spikes provided by solar activity could trigger the outburst.

Once the phase transition has been initiated, it will propagate within a region where the temperature of the ice is greater than 100 K. The volume into which the transition can propagate can be estimated. Our calculations consider a spherical comet with a radius of 5 km. For a rough estimate we assume that the surface temperature around the subsolar point decreases as a cosine function. For a subsolar temperature of 140 K, the surface area with a temperature above 100 K is bounded by a circle and contains 14% of the total surface area of the comet. Following Sommerfeld's¹¹ development, it is possible to estimate the depth at which the phase transition terminates 1 m below the subsolar point. For this calculation the thermal diffusivity of glass, 2×10^{-2} cm² s⁻¹, is used. As this depth is reduced to zero at the perimeter of the previously computed area, an average depth of 50 cm is assumed. The resulting volume which undergoes the phase transition is 2.2×10^{13} cm³. The density of amorphous ice is about 2 g cm⁻³ and so the mass involved is 5×10^{13} g.

The transformation of amorphous ice into cubic ice with a density of 0.94 g cm⁻³, must induce severe strains of the order of 20–30% which will pulverise the ice. It is useful to estimate the particle size which results from the fracturing process. For a stress, S , of about 10⁴ pound inch⁻², W is proportional to $S^{-1/2}$, where W is the average mass of the ten largest particles that result when the stressed material fractures. If this relationship holds in general for higher stresses, then an extrapolation to the anticipated stress encountered for the amorphous-cubic ice transformation ($\sim 10^6$ pound inch⁻² for strains of 20–30%), shows that W is of the order of 4×10^{-9} g. This implies that the largest particle has a size of about 10 μ m.

This mechanism therefore automatically provides a source of particulate matter which creates a huge surface area. Consequently, the equilibrium vapour pressure between the particulates will be rapidly established. With the mass (5×10^{13} g)

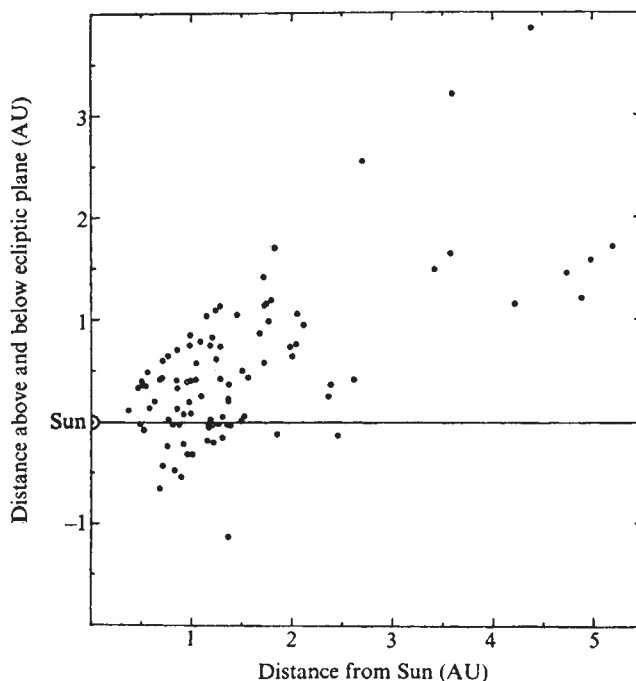


Fig. 1 Positions of comets at the time of outbursts. ○, Hypothetical outbursts preceding discovery; ●, observed outbursts after discovery⁹. (Courtesy of the IAU.)

involved in the phase transition the energy, E , which is released, is given by:

$$E = 5 \times 10^{13} \text{ g} \times 24 \text{ calorie g}^{-1} \\ = 10^{15} \text{ calorie}$$

This can generate water vapour of mass, M :

$$M = E/H = 10^{15} \text{ calorie}/650 \text{ calorie g}^{-1} \\ = 1.5 \times 10^{12} \text{ g}$$

where H is the heat of sublimation. This means that 3% of the generated particulate matter is sublimated. This gas must certainly expand into the vacuum of space at a few times the velocity of sound, carrying with it, at least to an order of magnitude, a comparable mass of dust. This mass is consistent with observed values for typical comet outbursts⁸.

A large fraction of the fractured material remains on the surface. This effectively creates an insulating layer with a high albedo which tends to prevent further outbursts for some time.

This picture of comet outbursts can be subjected to many refinements, such as variations of comet sizes, or of rotation rates and inclinations, impurities and inhomogeneities in the ice, and orbital parameters. The general features of this theory are, however, consistent with observations and in our opinion provide for a more plausible source of energy than has been previously suggested. Those suggestions have included the vaporisation of pockets of methane and/or carbon dioxide⁸, explosive radical reactions¹², and collisions with interplanetary boulders.

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HARVEY PATASHNICK

Dudley Observatory,
Albany, New York 12205

GEORG RUPPRECHT

Bendix Corporation,
Denver, Colorado 80236

DONALD W. SCHUERMAN

Dudley Observatory

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Ratio of the temperatures of the quiet Sun and the centre of the new moon at $\lambda = 1.3 \text{ mm}$

As radio wave spectral line observations are made at ever shorter wavelengths, the need for precise calibration increases. This is because, as a larger portion of the frequency spectrum becomes available for observation, an increasing number of multiple molecular transitions will be detected. To obtain reliable excitation parameters from these detections they must be calibrated accurately at each frequency. Linsky¹ has proposed the Moon as a radiometric standard for observations of extended sources in the infrared, millimetre and microwave portions of the spectrum. We therefore measured the ratio of the quiet Sun temperature to that of the centre of the new Moon

at 231 GHz ($\lambda = 1.3 \text{ mm}$).

The 36-foot telescope of the National Radio Astronomy Observatory (NRAO) was used for the measurements, on June 30, 1973. At $\lambda = 1.3 \text{ mm}$, using a 1.21λ by 1.77λ feed horn, the telescope has a beam size of $44'' \times 51''$ of arc as measured on Jupiter. A single ended mixer receiver was used that incorporated a Schottky-barrier diode as the nonlinear element². The noise temperature of the double-sideband receiver was 6,000 K, and the intermediate frequency bandwidth was 60 MHz, giving a ΔT r.m.s. of $\sim 1.5 \text{ K}$ with a time constant of 0.25 s.

At this frequency, antenna calibration is rather uncertain and atmospheric attenuation can be quite high, and so it was decided to carry out the observations when the Sun and Moon were close together in the sky. This allowed a comparison of brightness temperatures without any first order dependence on either the antenna or the atmospheric calibration. June 30, 1973 was the date of a total solar eclipse and during our measurements the centres of the Sun and Moon were separated by $6^\circ 45'$ in the sky: the elevation of the Sun was 41.7° and that of the Moon was 47.7° .

The absolute temperature scale of the receiver was calibrated by placing absorbers, alternately at room temperature and liquid nitrogen temperature, in front of the feed of the antenna. Sky dipping data gave an average value of $\sim 1.2 \text{ db}$ attenuation/atmosphere (clear weather; 27° C ; 35% relative humidity) which implied a differential attenuation of 0.18 db between the central solar and lunar positions. The Sun was observed through the dome of the telescope which had been previously measured (using the Moon) to have an attenuation of 2.87 db.

Antenna temperatures referred to an elevation angle of 41.7° , which, if corrected for dome and differential atmospheric attenuation would be 2,481.5 K and 53.2 K for the centres of the Sun and Moon respectively; a value of 46.6 for their ratio. Assuming that the temperature of the quiet Sun is 5,800 K at $\lambda = 1.3 \text{ mm}$ ³, a value of 124.5 K is derived for the central lunar temperature at new Moon.

An examination of H α and K spectroheliograms of the Sun taken on the day of our observations by the McMath-Hulbert Observatory, showed that except for a small flare close to the eastern limb of the Sun, the solar disk, particularly the central region where our observations were taken, was free from any major disturbance. This was confirmed by drift scans of the Sun during the observations. Thus, we are confident that the ratio quoted does refer to the quiet Sun.

Because of our method of observation, the value we have derived for the ratio of the quiet Sun to that of the new Moon is relatively insensitive to any errors in the absolute calibrations of the atmospheric attenuation, the antenna or the receiver. The value mainly depends on the linearity of the detector and the noise on the signal. Thus, we calculate that the error on the derived ratio—46.6—is ± 0.4 .

Bastin *et al.*³ quote the error on their solar measurement as $\pm 400 \text{ K}$. Consequently, the value we have derived for the central lunar temperature at new Moon—124.5 K—has an uncertainty of $\pm 8.6 \text{ K}$.

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G. T. WRIXON

University College
Cork, Ireland

M. V. SCHNEIDER

Bell Telephone Laboratories,
Holmdel, New Jersey 07733

Received May 20, 1974.

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Oldest and largest lunar basin?

MANY questions in lunar science have not yet been answered satisfactorily.

What, for example, is the origin of the nearside-farside asymmetry of the Moon? Why are Oceanus Procellarum and Mare Imbrium richer in KREEP (basalt rich in trace elements) than any other region on the Moon? Why are the lunar Apennines apparently not composed of anorthositic highland crust? I suggest here a possible solution to these, and other, problems.

The nearside-farside asymmetry is evident from the dark mare material which fills the circular basins on the nearside. Although there are similar basins on the farside, they are apparently not filled with mare basalt. Laser altimeter data suggest that this may be a result of the greater thickness of low density crust on the farside. The surface of the farside is largely elevated from a sphere of radius 1,738 km, centred on the Moon's centre of gravity, and the nearside is correspondingly depressed¹. Although this may explain why Maria occur only on the nearside, the differences in crustal thickness are still unaccounted for. Either the Moon accreted heterogeneously, or else low density crust was, at some early stage of lunar evolution, transferred from the nearside to the farside. A possible mechanism for the transfer of vast quantities of crustal material could involve a very major impact on the nearside.

Measurements with γ -ray spectrometers have revealed that the levels of natural radioactivity (which results from U, Th and K) are considerably higher in Oceanus Procellarum and Mare Imbrium than anywhere else on the lunar surface². These regions are therefore presumed to be richer in KREEP material. It is usually assumed that this distribution of KREEP arose as a result of the Imbrium impact. It has, however, been pointed out that if KREEP was ejected by the Imbrium event then the Haemus Mountains to the east of the Imbrium basin should be as radioactive as the Fra Mauro formation to the south³. This has not been found to be the case.

The age of the Imbrium event is generally taken to be about 3.9 Gyr (refs 4 and 5) whereas the whole rock 'isochron' ages for KREEP rocks are much older⁶, suggesting that KREEP basalts were extruded long before the Imbrium impact. The present day distribution of KREEP may, in fact, largely represent the extent of these original lava flows. The subsequent redistribution by the Imbrium impact may only have been a second order effect.

Before the Apollo 15 mission it had been expected that the lunar Apennines would consist of anorthositic crust, supposedly uplifted by the Imbrium impact. When samples were returned from the Apennine front, however, they were found to have predominantly a KREEP composition, and there were few truly anorthositic rocks. This lack of anorthositic crust in the region has since been confirmed by the results of the X-ray fluorescence experiments. The measured Al:Si ratio from the Apennines is about half the value obtained from the Descartes highlands⁷. These results suggest that the original crust in this region was removed and replaced with KREEP basalts, before the formation of the Imbrium Basin.

I propose that soon after the formation of the lunar crust the Moon collided with a planetesimal which was even larger than the projectile which formed the Imbrium Basin. This collision produced a basin 1,200 km in radius centred at 23°N 29°W (Fig. 1). Its size and position are defined by the highlands to the west of Oceanus Procellarum and to the north of Mare Frigoris, and by the western edge of the Southern Central Highlands. There is no evidence for the presence of any appreciable amount of anorthositic crust within this region. It must therefore have been largely removed, and at least some of it may even have been deposited on the lunar farside, con-

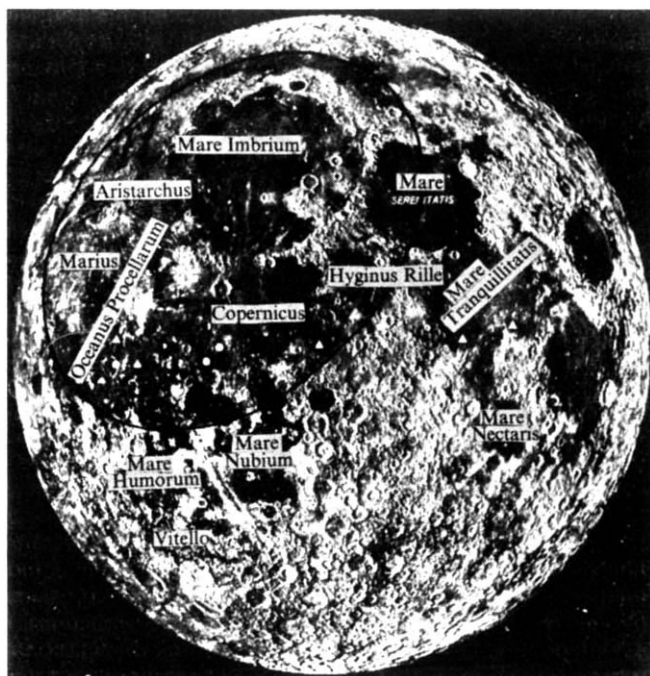


Fig. 1 The location of the 'Gargantuan Basin' on the lunar nearside.

tributing to the observed excess crustal thickness there. It is not unreasonable to suppose that this basin was subsequently filled with KREEP basalt lavas. This volcanic activity probably occurred about 4.3 Gyr ago; a date based on the whole rock 'isochrons' for Apollo 14 and 15 KREEP, and Apollo 16 VHA basalts⁶. Alternatively, the activity may have been as recent as 4.14 Gyr if only the Apollo 14 and 15 results are considered. About 3.9 Gyr ago the Imbrium impact redistributed these basalts, in the form of KREEP rich breccias, to the Fra Mauro formation and elsewhere^{4,5}. At the same time the lunar Apennines, largely composed of KREEP, would have been uplifted (see Fig. 2).

All of the large impact basins on the lunar nearside have circular, positive gravity anomalies, or mascons, associated with them⁸. The existence of the mascons, which are usually attributed to the presence of thick lenses of high density mare basalt, implies hydrostatic disequilibrium and provides a powerful constraint on any model of the thermal evolution of the Moon. The circular basin proposed here, however, does not have an associated mascon. If this basin was indeed filled with KREEP basalt 4.3 Gyr ago, in sufficient quantities to produce a mascon, the lunar crust at this time cannot have been rigid enough to support it. The absence of a mascon corresponding to this 'Gargantuan Basin' thus provides a very strong constraint for lunar thermal models.

If the Gargantuan Basin exists, the collision which produced it could have occurred before lunar capture by the Earth, during lunar capture, or after the Moon was in Earth orbit. One speculative possibility is that the Moon was originally in an eccentric solar orbit, possibly between Mars and Jupiter. Following a collision with a minor planet, that original orbit became perturbed into an orbit which crossed the Earth's, and the Moon was subsequently captured by the Earth. A second possibility is that the planetesimal which formed the basin was a minor terrestrial satellite which collided with the Moon as it passed near the Earth at a high velocity. Collision with a minor Earth satellite is a mechanism which could allow efficient lunar capture⁹. If the impact did indeed result in the capture of the Moon by the Earth, then the other large basins must all have formed when the Moon was in Earth orbit. Either the projectiles which produced them were themselves in

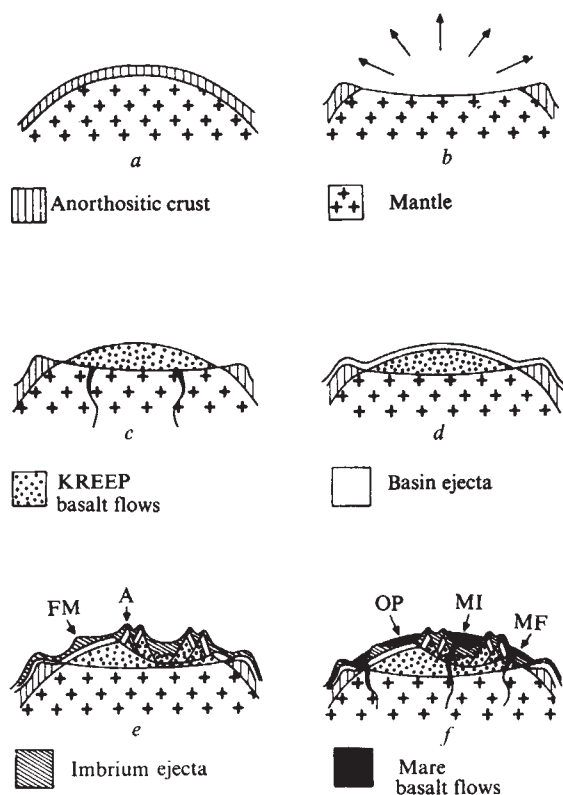


Fig. 2 The evolution of the lunar nearside: *a*, Differentiation of the lunar crust (4.6 Gyr); *b*, formation of the 'Gargantuan Basin', and deposition of excavated crust on the lunar farside; *c*, eruption of KREEP basalts (4.3 Gyr); *d*, deposition of ejecta from major basins (such as Serenitatis, 4.3–3.9 Gyr); *e*, deposition of the Fra Mauro formation (FM) and uplift of lunar Appennines (A) by the Imbrium impact (3.9 Gyr); *f*, eruption of mare basalts (3.8–3.2 Gyr). OP, Oceanus Procellarum; MI, Mare Imbrium; MF, Mare Frigoris.

Earth orbit and were collected as the Moon receded from the Earth because of tidal friction, or they may have constituted a family of asteroids in highly eccentric orbits which crossed the Earth's orbit. The inferred low impact velocity of the Imbrium projectile¹⁰ supports the first possibility. In either case it is difficult to explain why the projectiles were not all finally accreted until at least 400 Myr after lunar capture.

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P. H. CADOGAN

Department of Physics,
The University, Sheffield, UK

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Can Einstein's theory of gravitation be tested beyond the geometrical optics limit?

AN upper limit of a few $\times 10^{-3}$ arc s has been placed¹ on the differential deflection of right and left circularly polarised (r.c.p. and l.c.p., respectively) radiation which passes by the Sun from the quasar 3C273. The separation of quasar images in r.c.p. and l.c.p. radiation permits a measurement of the effect of gravitation on polarised radiation. This seems to be the first experiment in which Einstein's theory of gravitation is tested beyond the geometrical optics approximation. Here, I estimate the new effect (that is, the differential gravitational deflection of polarised radiation), and point out that though, using present techniques, it is too small to be measurable for the Sun, it is, however, potentially observable for gravitationally collapsed objects.

Electromagnetic waves in a gravitational field can be thought of as propagating in flat spacetime but in the presence of a 'medium' with properties which are given by conformally invariant quantities constructed from the metric tensor of the Riemannian spacetime, in Cartesian coordinates (ref. 2). Here, Greek indices run from 0 to 3, and Latin indices from 1 to 3. Gravitational units, in which $G=c=1$, are used throughout. The electromagnetic field tensor is decomposed in a Cartesian frame, $(t=x^0, \mathbf{x}_i=x^i)$ $F_{\mu\nu} \rightarrow (\mathbf{E}, \mathbf{B})$, $(-g)^{1/2} F^{\mu\nu} \rightarrow (-\mathbf{D}, \mathbf{H})$ to obtain the usual Maxwell's equations in a medium, together with the relationships²

$$\mathbf{D}_i = \epsilon_{ij} \mathbf{E}_j - (\mathbf{G} \times \mathbf{H})_i \quad (1)$$

$$\mathbf{B}_i = \mu_{ij} \mathbf{H}_j + (\mathbf{G} \times \mathbf{E})_i \quad (2)$$

where, $\epsilon_{ij} = \mu_{ij} = -(-g)^{1/2} g_{00}$, and $G_i = -g_{0i}/g_{00}$. If the vectors $\mathbf{F}^\pm = \mathbf{E} \pm i\mathbf{H}$ and $\mathbf{S}^\pm = \mathbf{D} \pm i\mathbf{B}$, $S_i^\pm = \epsilon_{ij} F_j^\pm \pm i(\mathbf{G} \times \mathbf{F}^\pm)_i$ are introduced, then the equations

$$(1/i) \nabla \times \mathbf{F}^\pm = \pm (\partial/\partial t) \mathbf{S}^\pm \quad (3)$$

$$\nabla \cdot \mathbf{S}^\pm = 0 \quad (4)$$

determine the properties of waves in a gravitational field³. The coordinate transformation, $t' = t + \phi(\mathbf{x})$, induces the gauge transformation, $G'_i = G_i + \partial\phi/\partial x^i$, and $\epsilon'_{ij} = \epsilon_{ij}$, under which $\mathbf{F}^\pm$ remains invariant, $\mathbf{F}'^\pm(t', \mathbf{x}) = \mathbf{F}^\pm(t, \mathbf{x})$, a property which will be used later. $\mathbf{G}$ and $U_{ij} = \delta_{ij} - \epsilon_{ij}$, can be interpreted as gravitational vector and tensor potentials, respectively.

In the scattering of electromagnetic radiation from a gravitational field (asymptotically flat spacetime with no other electromagnetic fields present) the r.c.p. radiation ($\mathbf{F}^- = 0$, polarisation properties determined at infinity) decouples from l.c.p. radiation ($\mathbf{F}^+ = 0$) according to equations (3) and (4). The helicity of the photon is thus conserved. Moreover, if the gravitational field is free of matter, and spherically symmetrical, then the scattering amplitude is independent of the photon helicity and the gravitational field does not alter the state of polarisation of the incident wave. For more general fields, such as the Kerr field or the exterior field of a rotating body, the scattering amplitude is dependent upon the helicity. The physical reason for this is easily seen by writing the equation for waves of frequency ω in the exterior field of a slowly rotating body of mass, m , and angular momentum, $\mathbf{J} = J\hat{z}$:

$$[(1/i) \nabla - \omega \mathbf{G}] \times \mathbf{F}^\pm = \mp i\omega N \mathbf{F}^\pm \quad (5)$$

where, $\mathbf{F}^\pm = \mathbf{F}^\pm \exp(-i\omega t)$; $\mathbf{G} = 2\mathbf{J} \times \mathbf{r}/r(r-m/2)^2$; and $N = (1+m/2r)^2/(1-m/2r)$. The index of refraction, N , causes a general deflection for any wave packet⁴. The photon interacts with the vector potential $\mathbf{G}$; not only with its gravitational 'charge' (energy) but also with its spin, because a rotating body produces a gravitational 'magnetic' field. Moreover, $r \gg m$, $G \approx 2 \mathbf{J} \times \mathbf{r}/r^3$, which is the vector potential caused by a gravitational 'magnetic moment' $2\mathbf{J}$. The spin of the

photon is parallel (or antiparallel) to its momentum, and therefore the path of a photon in the exterior field of a slowly rotating body depends on its helicity. It should be emphasised that in the geometrical optics limit where terms of the form $\lambda |\partial g_{ij} / \partial x_k|$ are neglected, the rays follow

$$(\nabla \mathbf{S})^2 - 2\omega \mathbf{G} \cdot \nabla \mathbf{S} = \omega^2 N^2 \quad (6)$$

regardless of their polarisation. Thus, wave packets with different polarisation states are expected to follow separate paths which differ from equation (6) in the first approximation by terms of the order of $\lambda |\partial G_i / \partial x_j|$, or the amount by which the vector potential varies over the wavelength of the radiation.

The path of a wave packet of frequency ω , $\omega m \gg 1$, moving nearly parallel to the y axis in the y - z plane far away from the rotating object, is expected to be influenced by the gravitational field of the object at distances close to D along the z axis, where $G \approx \eta \times 2r$, $\eta = 2J/D^3 \ll 1$ and $N \approx 1 + 2m/r$. The dimensions of the packet are assumed to be very small compared with D . The new effects are connected with the presence of G , it is therefore of interest to consider the solutions of the wave equation

$$[(1/i)\nabla - \eta \hat{z} \times \mathbf{r}] \times \Phi^\pm = \mp i\omega \Phi^\pm \quad (7)$$

which, under the gauge transformation $\Psi^\pm = \Phi^\pm \exp(i\omega \eta xy)$, is transformed into

$$[(1/i)\nabla - \omega \mathbf{A}] \times \Psi^\pm = \mp i\omega \Psi^\pm \quad (8)$$

with $A_x = A_z = 0$, and $A_y = 2\eta x$. Solutions of equation (8) can be found in the form $\Psi^\pm = \Psi^\pm(x) \exp(iK_y y + iK_z z)$, together with the dispersion relation

$$\omega^2 = (K_z^\pm)^2 \pm 2\eta K_z^\pm + 2\eta \omega (2n^\pm + 1) \quad (9)$$

where, $n^+ = 0, 1, 2, \dots$. This relationship implies that for r.c.p. and l.c.p. photons in the same state, Ψ , $n^+ = n^-$, and moving in essentially the same directions:

$$K_z^- - K_z^+ = 2\eta \quad (10)$$

Thus, the paths of r.c.p. and l.c.p. photons propagating in a direction other than the direction of rotation, split because of the interaction of the photon helicity with the angular momentum of the body, as is evident from equation (9). If the waves propagate along the direction of rotation of the body then equation (10) amounts to different indices of refraction for r.c.p. and l.c.p. waves, so that the plane of linear polarisation rotates in the same direction as the body, in agreement with the results of Skrotskii⁵. Therefore, the wave packet under discussion is focused differently, depending on its state of polarisation, and the differential deflection angle is given by

$$\delta = \omega^{-1} |K_z^+ - K_z^-| = \gamma \lambda J / D^3 \quad (11)$$

where $\lambda = 2\pi/\omega$ and γ is a numerical factor which is expected to be of the order of unity.

Harwit *et al.*¹ used 8 GHz radio waves which passed by the Sun at a distance of $\sim 7.8 R_\odot$. For the Sun, $\delta_\odot < 10^{-18}$ arc s, which is too small to be detectable, even with the improved techniques of very long base line interferometry, which are likely in the near future. The effect is, however, potentially observable for collapsed objects, and may provide a means of measuring the angular momentum of the body.

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BAHRAM MASHHOON

Joseph Henry Laboratories,
Princeton University,
Princeton, New Jersey 08540

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Q and mantle creep

EXPERIMENTS on model materials show that the acoustic properties of the low velocity zone (LVZ) can arise from the formation of intergranular fluid or melt with no significant reduction in creep strength. Consequently, only very limited bounds on the asthenosphere can be inferred from seismic data.

The effect of partial melting on the acoustic velocity, v , and attenuation, Q^{-1} , of a material is sensitive to the dihedral angle, θ , of the melt in the solid grain boundaries. Relevant data are summarised in Figs 1 and 2. When $\theta = 0$, the melt wets the grain boundaries and a small volume fraction of fluid is sufficient to decrease greatly v and Q^{-1} . There is no theory which accounts for this. In the interpretation of seismic data, however, $\theta = 0$ is the case of greatest interest because the glass formed by quenching samples of partially melted crystalline rock always wets the grain boundaries. The volume fraction of melt required to account for the LVZ is estimated from the data (Figs 1 and 2) to be about 0.01.

In a multicomponent system with a solidus temperature T_s , and liquidus temperature, T_l , partial melting occurs when $T_s < T < T_l$. The presence of a small quantity of fluid at a temperature above T_s means that grain boundaries no longer offer resistance to plastic deformation and that a liquid path is available for diffusion. If deformation because of diffusion through the fluid is not rate-controlling, the creep strength of a partially melted polycrystal in the absence of tensile stress is hardly affected by the fluid. The creep strength reflects the strength of the individual solid grains, and depends on T/T_m , where T_m is the melting temperature of the pure solid. It should, then, be possible to put a material in which T_m/T_s is large, and $\theta = 0$, in a state of partial melting such that the acoustic attenuation and creep strength are both large and the sound velocity is low.

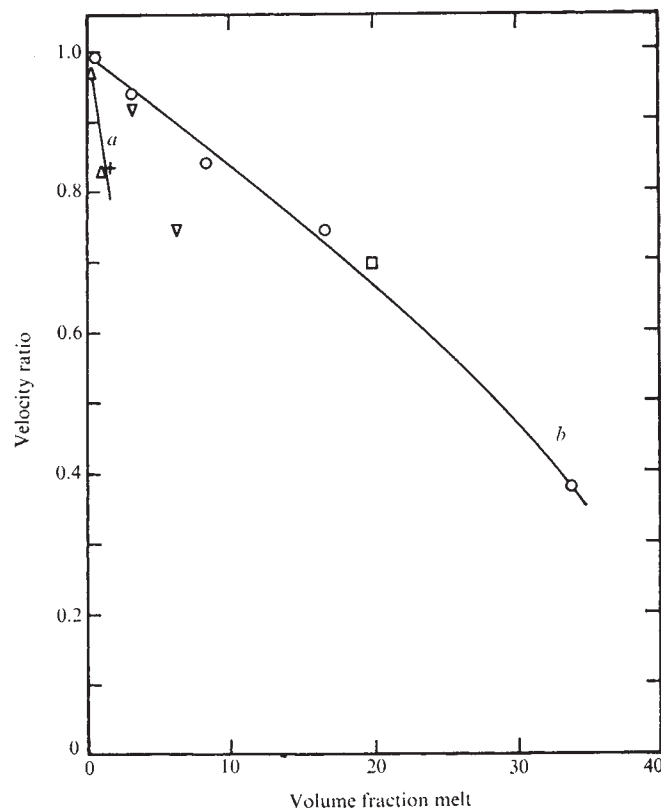


Fig. 1 Ratio of extensional wave velocity in partial melts at the solidus to that in the solid phase, as a function of the volume fraction of melt. *a*, $\theta = 0^\circ$; *b*, $\theta = 60^\circ$. Materials are: $\square$, W-Ag (ref. 6); $\circ$, Cu-Pb (ref. 7); inverted Δ , NaCl-H₂O (ref. 8); +, KCl-AgCl (ref. 9); Δ , Cu-Ag (ref. 7).

This hypothesis has been tested with experiments on an Al-2 at % Ga alloy. In this alloy $T_s = 29^\circ\text{C}$ and $\theta = 0$; T_m for pure aluminium is 660°C . Above 29°C the alloy contains about 1% melt; it has no tensile strength and can be separated easily into individual grains, each coated by a liquid film. The velocity and attenuation of sound in the Al-Ga alloy have been measured above and below T_s using the method of driven extensional resonance (Table 1). When T_s is exceeded the attenuation increases by a factor of 40 and the velocity drops by 4%. Creep strength measurements have been made on bars of the same alloy loaded in compression at constant stress (Fig. 3). Initially, there is a rapid decrease in strain rate, $\dot{\epsilon}$, identified with transient creep. When steady state creep has been established, after about 10 h at 20°C , $\dot{\epsilon}$ decreases at a constant rate because of the increase in cross section that results from compression. In the experiment illustrated, the temperature was increased from 20° to 33°C after the test had run for 38 h. There was a transient increase in $\dot{\epsilon}$, but after 10 h the creep rate had again become steady and was about the same as that of the alloy containing no melt. Partial melting resulted in no increase in the steady state creep rate.

Table 1 Acoustic properties of Al-Ga alloys

Temperature ($^\circ\text{C}$)	Velocity (km s $^{-1}$)	Attenuation (Q^{-1})
22	5.35	1.0×10^{-4}
32	5.13	39×10^{-4}

If a material is to offer little resistance to steady state creep deformation, then $T \rightarrow T_1$. If T_1 is much less than T_m the small resistance arises because there is enough fluid to disaggregate the solid structure; if $T_1 \rightarrow T_m$ the grains of the solid have an intrinsically low creep resistance, regardless of the detailed nature of the operative creep mechanism¹. It is not necessary that $T \rightarrow T_1$ for the acoustic attenuation to be high and the sound velocity anomalously low. These acoustic characteristics can be produced either by heating slightly above T_s when $(T_1 - T_s)/T_1$ is large, or, in rock, by the presence of

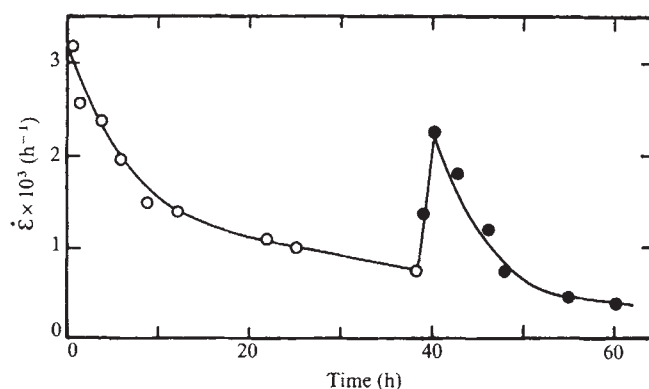


Fig. 3 Creep rate of an Al-Ga alloy under constant compressive load, initially at 20°C , and after 38 h, at 33°C .

small quantities of intergranular fluid rich in water. (Open cracks can also cause the acoustic characteristics of the LVZ but are unlikely to occur under high hydrostatic pressure.) The existence of a LVZ is not, therefore, evidence for the presence of an asthenosphere. A gradual variation of mantle strength properties with depth, as predicted on the basis of solid state data²⁻⁴, is fully consistent with the seismic structure of the upper mantle.

Seismic data fail to establish bounds for either the temperature or the strength properties of the upper mantle. They can, however, place a bound on the properties of the lower mantle. All polycrystalline solids with acoustic properties which have been tested to high temperature in the laboratory show a rapidly increasing 'background' internal friction as $T \rightarrow T_m$ (ref. 5). This is thought to be a result of the coupled, stress-induced motion of dislocations and solute atoms, but conclusive evidence is lacking. In order for the acoustic attenuation of the lower mantle to be as low as the observed value of 10^{-3} , the temperature must be below that at which significant background internal friction appears. Comparison with laboratory data on the high temperature background internal friction of polycrystalline ceramics indicates that in the lower mantle, $T < (0.5-0.75)T_m$.

THOMAS A. AUTEN*
ROBERT B. GORDON
RICHARD L. STOCKER†

Department of Geology and Geophysics,
Yale University,
New Haven, Connecticut 06520

* Present address: Department of Mechanical Engineering, Iowa State University of Science and Technology, Ames, Iowa 50010.

† Present address: Lehigh University, Bethlehem, Pennsylvania 18015.

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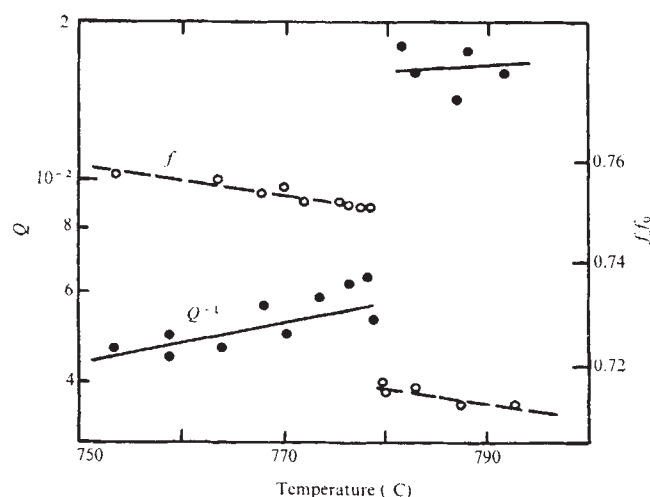


Fig. 2 Internal friction, Q^{-1} , and frequency of extensional resonance relative to that at room temperature, f/f_0 , for a Cu-8.13 weight % Ag alloy heated through T_s (ref. 7). At T_s there is a discontinuous change in both Q^{-1} and the frequency ratio. The volume fraction of liquid formed is 1.4×10^{-3} .

Origin of iron ore by diagenetic replacement of calcareous oolite

MANY non-uniformitarian¹ or quantitatively improbable² hypotheses have been offered to explain the origins of extensive sedimentary rocks rich in iron. Ironstones and iron formations³, here termed non-cherty and cherty iron ores, have, however, two consistent characteristics which suggest that their origins were fundamentally similar and involved processes which are still operative at present⁴. The first is the occurrence of mudrocks, or their metamorphosed equivalents, overlying individual ore beds within a few metres, with the ore beds themselves covered by non chemical sedimentary rock and not eroded. The second is the occurrence of oolite texture in unmetamorphosed deposits which are not closely associated with volcanic rock or shales bearing pyroclastic material. The internal structures of well preserved ferriferous ooids closely resemble those of Recent calcareous ooids. Some Recent ferriferous spherules are concentrically layered⁵ but, unlike the ooids in iron ores, they contain clay minerals other than ferrous septechlorite, and occur collectively only as moderately iron-rich sediments, no thicker than a few tens of centimetres. Extensive beds of ferriferous oolite thicker than 1 m have formed repeatedly throughout the history of the Earth, even within the last 5 Myr (ref. 6).

The problem is how can the precipitation from solution of major quantities of iron and silicon, with or without substantial aluminium, phosphorus and manganese, give rise to extensive beds which contain ferrous minerals and shallow water sedimentary structures. A study of the mid-Cainozoic Paz de Rio iron ore of north-eastern Columbia⁴ has resulted in a genetic model which is most clearly illustrated in reference to Upper Pliocene ore under, and adjacent to, the Sea of Azov (Fig. 1).

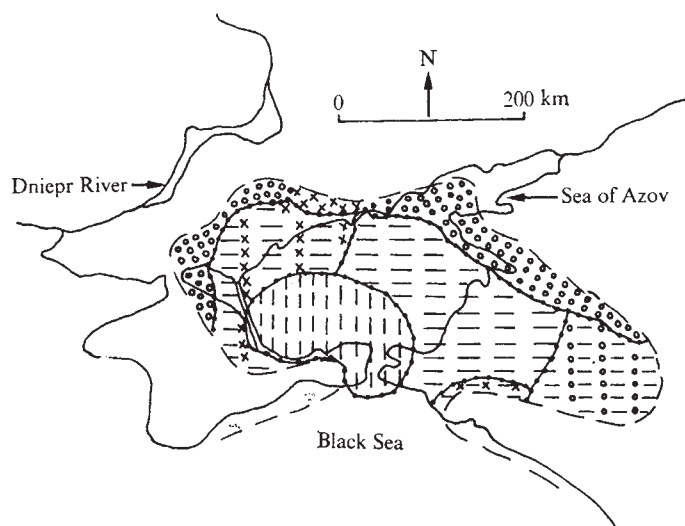


Fig. 1 Map 20 of Upper Pliocene lithofacies in the former Sea of Azov. Circles, non-ferruginous sand; crosses, ferruginous sand; horizontal dashes, shale; vertical dashes, ferriferous oolite with overlying shale. Interbedding is represented by alternation of symbols.

Like all other non-cherty ores, the Azov deposit is predominantly oolitic, and the average thickness of continuous iron ore is only a few metres, although thicknesses do reach 52 m locally. The most abundant ore mineral is ferrous, septechlorite rich in aluminium (chamosite), which is partially replaced by siderite⁶. Goethitic oolite has formed by the oxidation of chamositic oolite above the present water table in the Kerch Peninsula. Fossil shells and shell molds are common, and barite pseudomorphs of wood are present locally. Soviet investigators have suggested that one

possible origin is the dissolution resulting from bacterial action, of terrigenous sediment on the seafloor, and the precipitation of the derived solutions as chamositic oolite at the sediment-water interface⁶. The concentrations of dissolved aluminium in modern marine water⁷, 0.001 parts per million (p.p.m.), and fluviolacustrine water⁸, < 1 p.p.m., are, however, too low to supply sufficient quantities to an extensive region of hypothetical chamositic sedimentation. There is more aluminium in the Azov ore chamosite⁶ than there is dissolved in all of the oceans of the world. Moreover, the absence of dissolved oxygen, which is required if ferrous iron is to be rendered soluble, is incompatible with transport in very shallow, turbulent water.

I therefore propose that the iron, aluminium, and silicon were dissolved by organic, acid-rich groundwater from the interdistributary clay of a delta which prograded over calcareous oolite deposits. Like calcareous oolite sediment in the modern Caspian and Aral Seas⁹, Upper Pliocene oolite in the Sea of Azov apparently formed far from major river discharge and was circumvented by terrigenous sediment (Fig. 1). Shale covering the oolite is interpreted as representing subsequent deltaic progradation. Rain and flood water trapped between natural levees on the delta would have drained from interdistributary swamps rich in organic material, through the highly permeable oolite, up to distributaries (Fig. 2). Aluminium would have been transported in solution as metallo-organic complexes at concentrations of between one and a few p.p.m., as in some British bogs¹⁰. This has been done experimentally at room temperature in 0.01M humic acid solution of clays¹¹. Organic complexing would have greatly enhanced the solubility of iron, but not that of silicon. The experimentally determined Al:Si molar ratio for solutions of clay minerals in dilute, strongly complexing acids, is close to unity¹¹, similar to the molar ratio in chamosites⁴.

If only free ferric oxide and amorphous aluminosilicates were dissolved under interdistributary swamps, over 20 times (ref. 12) as much deltaic mud as oolite would have been leached before the completion of replacement. Migrating distributaries would have continually eroded weathered silt and clay toward the Black Sea. The duration of replacement may be estimated by assuming a deltaic sedimentation rate of 50 million tons a year (about one-tenth that of the Mississippi River) on to the area of oolite illustrated in Fig. 1, and leaching of a volume of deltaic sediment 30 times greater than that of the oolite replaced. Alternatively, the duration can be estimated using Darcy's Law, by assuming the precipitation of 20 p.p.m. of iron from groundwater, a permeability of 100 darcy for oolitic sediment, and a head loss of 1m between interdistributary swamps and distributary channels (Fig. 2). Both calculations suggest that roughly 10⁵ yr are required for the replacement of 5 m of oolite⁴.

The dissolution of silicates by solutions rich in organic material and the subsequent replacement of calcareous oolite, have been experimentally simulated by Gruner², who found that internal ooid structures were preserved in the ferriferous replacement. The preservation of the internal structure is here attributed to the presence of primary carbonaceous layers within calcareous ooids, which act like templates, as in the replacement of aragonite by calcite¹³. Oxidic ooid layers which alternate with chamositic layers in well preserved, mid-Cainozoic ore in Colombia, formed by the oxidation of selected chamositic layers⁴, probably because of differences in the content of organic matter. The greatest loss of oolitic texture in this ore has occurred at the top, possibly as a result of initial rapid dissolution by descending groundwater.

Those who oppose the idea that ferriferous oolite may have originated by the replacement of calcareous oolite, have noted the common occurrence of unreplaced fossil shells beside ferriferous ooids in Phanerozoic ores. Recent

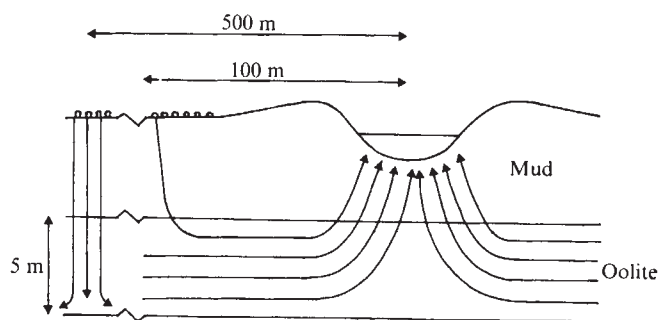


Fig. 2 Hypothetical cross section of groundwater flow from an interdistributary swamp through oolite to a distributary.

oids are, however, relatively porous aggregates of minute, aragonitic crystallites¹³ which would react readily. Gruner² found that small, calcareous shells were not replaced by solutions which replaced thin sections of calcareous oolite. Opponents to the idea have also noted the lack of lateral gradation from ferriferous oolite to calcareous oolite. But deltaic sedimentation sufficiently potent to fill a lagoon landward from a shallow bank of calcareous oolite, like that in the Aral Sea⁹, would readily cover the entire surface of an oolite bank and cause uniform replacement.

Chamositic oolite has been forming for at least the past 2×10^9 Myr (ref. 14) and cherty, ferriferous oolite has formed at least as recently as the early Jurassic¹⁵. Lower Proterozoic chamositic iron ore is interpreted as recording organic complexing in groundwater which drained interdistributary swamps. The common association of cherty, iron ores poor in aluminium³, of all ages, with volcanic rock or shale containing pyroclastic material¹⁶ suggests that much of the silica and iron was leached from the overlying pyroclastics. A lesser degree of organic complexing during rapid weathering of pyroclastics would have resulted in the decreased solubility of aluminium, whereas iron would have been relatively soluble in a reducing environment under swamps.

The ratio of iron ore to other sedimentary rock is typically very high in Lower Proterozoic ore-bearing sequences, although the total thicknesses within the Phanerozoic of some shallow water sedimentary sequences which include thin ore beds, are comparable to those of the Lower Proterozoic^{4,17}. The difference in the proportion of iron ore is attributed to a difference in the proportion of non-shelly, calcareous sedimentation within slowly subsiding, shallow, restricted basins like the Sea of Azov. Thick ores are attributed to cyclical transgressions of oolite over exposed areas of pyroclastics and/or deltaic sediments which were largely or entirely eroded by fluvial action and marine transgression. Non-calcareous sedimentation unaccompanied by basin subsidence may have lasted longer than calcareous sedimentation without leaving much record.

Cherty ores display a wide variety of textures and structures. Layers of ferrous septeclorite poor in aluminium (greenalite) in some siliceous ooids from the Lower Proterozoic Gunflint Formation of Ontario, are as fine and regular as aragonitic layers in Recent ooids. Elsewhere, the oolitic texture has been partially destroyed during the diagenetic development of compositionally distinct bands¹⁸. Banding is characteristic of most cherty oolite, even where it is non-ferriferous¹⁹, and must be related to the process of silicification. Laterally similar properties of the primary calcareous sediment may have permitted the planar flow of groundwater from which laterally continuous chert layers precipitated.

I therefore conclude that ferriferous oolite is calcareous oolite that has been covered by deltaic sediment and/or pyroclastics which were exposed to weathering and leached.

Water, rich in solutes, drained laterally through the underlying oolite, replacing aragonitic ooids by chamosite if it was rich in aluminium, or by greenalite and chert if it was poor in aluminium. I predict that oxygen isotopic ratios of chamosite in marine ores will indicate precipitation from fresh water. Extensive ferriferous oolite could readily form within the next 10^6 yr by the diagenetic replacement of calcareous oolite in the north-western portion of the Aral Sea⁹.

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M. M. KIMBERLEY

Erindale College,
University of Toronto,
Mississauga, Ontario L5L 1C6,
Canada

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In vitro transcription of three adjacent *E. coli* transfer RNA genes

RECENT evidence suggests that the structural genes of some transfer RNA molecules are clustered in groups of two or three on the *Escherichia coli* chromosome¹⁻⁵. The arrangement of tRNA and spacer sequences for two such tRNA clusters have been described⁶⁻⁸. The pattern of transcription of tRNA genes in clusters is not known although the formation of larger precursors in tRNA biosynthesis has been reported^{9,10}. *In vitro* transcription of tRNA₁^{Tyr} gene carried by $\phi 80\text{psu}^+$ phage DNA by purified RNA polymerase was shown to result in the formation of pre-tRNA^{Tyr} molecules bigger in size than mature tRNA¹¹. In the present communication we describe the transcription of a tRNA gene cluster (tRNA₂^{Gly}(su⁺), tRNA₂^{Tyr}, tRNA₃^{Thr}) carried by the DNA of transducing phage $\lambda\text{h80Cl857S}^+\text{t68dglyTsu}^+\text{tyrTthrT}$ (abbreviated λh80T) recently isolated by Squires *et al.*⁵. *In vitro* transcription of this phage DNA by purified RNA polymerase is shown to produce pre-tRNA molecules of a molecular size consistent with the model of a polycistronic precursor.

The defective transducing phage λh80T was prepared according to Squires *et al.*⁵ from a double lysogen of strain BF 266. Separation from the helper phage $\lambda\text{h80Cl857S}^+\text{t68}$ (λh80H) was carried out by equilibrium density centrifugation.

E. coli ³²P-tRNA was annealed to λh80T phage DNA in the presence of increasing amounts of unlabelled *E. coli* tRNA, purified tRNA^{Tyr} or a tRNA preparation devoid of tRNA^{Tyr}.

The hybridisation-competition curves presented in Fig. 1 show that unlabelled *E. coli* tRNA competes efficiently with the *E. coli* (^{32}P) tRNA, reducing its hybridisation almost completely. On the other hand, purified tRNA^{Tyr} and a tRNA preparation lacking tRNA^{Tyr} reduce ^{32}P -tRNA hybridisation by only 32 and 65% respectively. These data are in accord with the tRNA gene content described for the λ h80T phage DNA⁵. Due to the presence of three tRNA genes, tRNA^{Tyr} is expected to compete with only one third of ^{32}P -tRNA hybridisation to the phage DNA whereas a tRNA preparation lacking tRNA^{Tyr} should compete only with the hybridisation of the two other RNA species.

Phage λ h80T DNA was transcribed *in vitro* by DNA-dependent RNA polymerase in the presence or absence of ρ termination factor. In order to follow the transcription of the tRNA genes carried by λ h80T DNA we examined the ability of the *in vitro* synthesised RNA to compete with *E. coli* ^{32}P -tRNA for hybridisation sites on the phage DNA. An annealing experiment was carried out using a constant amount of λ h80T DNA and *E. coli* ^{32}P -tRNA in the presence of increasing amounts of *in vitro* synthesised λ h80T RNA. The results presented in Fig. 2a show that the *in vitro* synthesised λ h80T RNA contains polynucleotide chains homologous to those of *E. coli* tRNA. These results also imply that all three tRNA genes carried by λ h80T DNA were transcribed *in vitro* since the synthesised RNA competes efficiently with more than 90% of the hybridisation of *E. coli* ^{32}P -tRNA to λ h80T DNA. The specific production of tRNA₂^{Tyr} sequences was demonstrated using

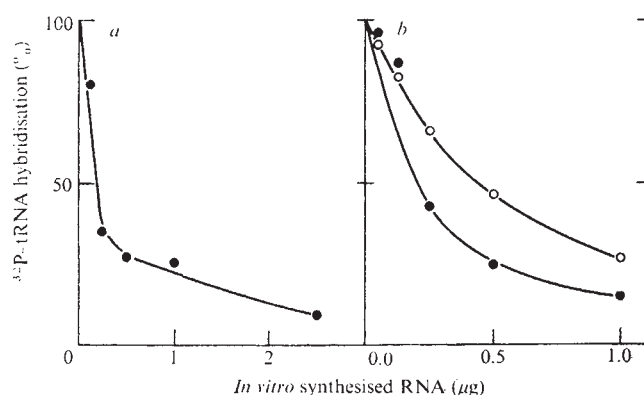


Fig. 2 a, Competition between *in vitro* synthesised λ h80T RNA and *E. coli* ^{32}P -tRNA for hybridisation with λ h80T DNA. λ h80T DNA was transcribed *in vitro* by *E. coli* RNA polymerase in the presence of ρ factor. DNA-dependent RNA polymerase was prepared from *E. coli* MRE-600 cells¹⁴ and further purified by low salt glycerol gradient centrifugation¹⁵. Termination factor ρ was prepared from *E. coli* MRE-600 cells as described by Roberts¹⁶. Transcription was carried out in a reaction mixture containing in 10 ml: 50 μmol Tris HCl (pH 7.9); 10 μmol MgCl_2 ; 1 μmol dithiothreitol; 75 μmol KCl; 0.4 μmol each of ATP, GTP, UTP and ^3H -CTP (4×10^3 c.p.m. nmol^{-1}); 300 μg of λ h80T DNA; 760 units RNA polymerase ($2,300 \text{ U mg}^{-1}$) and 65 μg ρ factor. The synthesis was initiated, after 5 min pre-incubation at 38°C , by the addition of the nucleoside triphosphates. The reaction was stopped and RNA isolated as described elsewhere¹¹. The hybridisation mixture contained, in a volume of 0.5 ml $2 \times \text{SSC}$, 10 μg denatured λ h80T DNA, 0.9 μg ^{32}P -tRNA (8.5×10^5 c.p.m. μg^{-1}) and increasing amounts of *in vitro* λ h80T RNA. The mixtures were incubated for 90 min at 70°C , loaded on filters and processed as described in legend to Fig. 1. The radioactivity remaining on the filters was expressed as percentage of control which contained no competing RNA. b, Competition between *in vitro* synthesised λ h80T RNA and *E. coli* ^{32}P -tRNA for hybridisation with $\phi 80\text{psu}_3^+$ L strand DNA. λ h80T DNA was synthesised *in vitro* by transcribing λ h80T DNA with RNA polymerase holoenzyme (O—O) or holoenzyme + ρ factor (●—●). $\phi 80\text{psu}_3^+$ DNA preparation and strand separation were as previously described¹¹. The hybridisation mixture contained in 0.3 ml $2 \times \text{SSC}$, 2 μg $\phi 80\text{psu}_3^+$ L strand DNA, 0.9 μg ^{32}P -tRNA and increasing amounts of *in vitro* synthesised λ h80T RNA. The mixtures were incubated for 90 min at 70°C , loaded on filters and processed as described above.

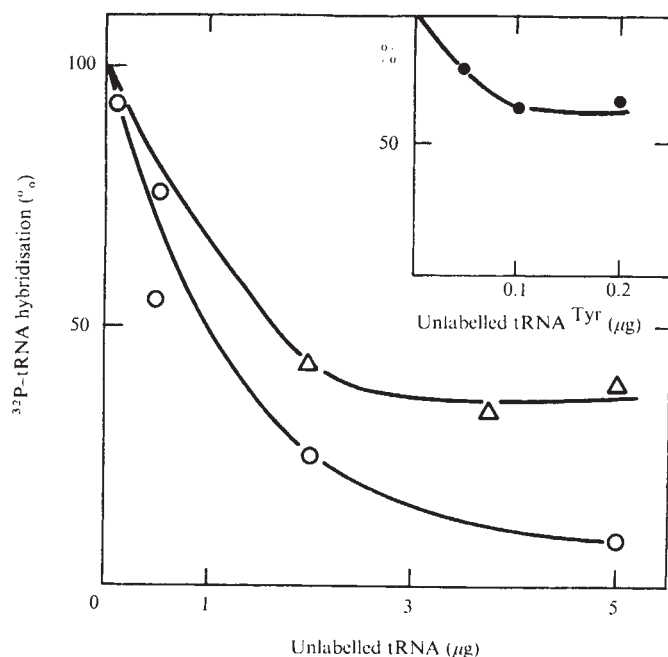


Fig. 1 Competition of *E. coli* ^{32}P -tRNA with unlabelled *E. coli* tRNA, tRNA^{Tyr} and tRNA devoid of tRNA^{Tyr} for hybridisation to λ h80T DNA. *E. coli* tRNA and ^{32}P -tRNA were prepared as described previously¹². tRNA^{Tyr} was prepared by fractionation of N-carbobenzoyloxy-(^{14}C)-tyr tRNA on a methylated albumin-silicic acid column¹³. The tRNA lacking tRNA^{Tyr} (containing only 10% of the original tRNA^{Tyr}) was eluted from such a column at low salt concentrations while the N-carbobenzoyloxy-(^{14}C)-tyr tRNA was eluted with 1 to 2 M NaCl. λ h80T DNA was prepared as previously described¹¹. Nitrocellulose filters containing 3.5 μg of denatured λ h80T DNA were immersed in hybridisation mixtures of 0.3 ml $2 \times \text{SSC}$ (0.3 M NaCl, 0.03 M Na citrate) containing 1 μg of *E. coli* ^{32}P -tRNA (1.2×10^6 c.p.m. μg^{-1}) and increasing amounts of unlabelled *E. coli* tRNA (O—O), tRNA devoid of tRNA^{Tyr} (Δ — Δ) and tRNA^{Tyr} (●—●). After 18 h at 67°C the filters were removed, washed with $2 \times \text{SSC}$, treated with pancreatic RNase (25 $\mu\text{g ml}^{-1}$ in $2 \times \text{SSC}$), washed again and counted. The radioactivity remaining on the filters was expressed as percentage of control which contained no competing RNA.

the $\phi 80\text{psu}_3^+$ DNA. This transducing phage DNA carries on the L strand the tRNA₁^{Tyr} gene^{11,17} which differs in sequence from tRNA₂^{Tyr} gene by only two nucleotides¹⁸. The difference in sequence between tRNA₁^{Tyr} and tRNA₂^{Tyr} being very small, we assumed tRNA₂^{Tyr} to hybridise equally well to the tRNA₁^{Tyr} gene carried by $\phi 80\text{psu}_3^+$ DNA. Accordingly, we followed the *in vitro* synthesis of tRNA₂^{Tyr} on λ h80T DNA by its competition with *E. coli* ^{32}P -tRNA hybridisation to the L strand of $\phi 80\text{psu}_3^+$ DNA Fig. 2b. These results establish the presence of tRNA₂^{Tyr} chains among the tRNA-like polynucleotides synthesised *in vitro* on λ h80T DNA.

λ h80T RNA transcribed *in vitro* in the presence or absence of ρ factor was fractionated by centrifugation through a sucrose gradient. Figure 3 shows that the total RNA synthesised *in vitro* has a wide size distribution and that transcription of λ h80T DNA in the presence of ρ results in size reduction of RNA molecules having sedimentation constants of 16S or higher. The presence of tRNA₂^{Tyr}-like molecules along the gradient was determined, as described in Fig. 2b, by competition with *E. coli* ^{32}P -tRNA hybridisation to the L strand of $\phi 80\text{psu}_3^+$ DNA. The histograms presented in Fig. 3 show the distribution of the tyrosine specific tRNA-like chains in the gradient fractions. The tRNA₂^{Tyr}-like chains seem to be located in two main fractions (2 and 3) on the gradient and to possess sedimentation values higher than 4S. Once again it is observed that

the presence of ρ during transcription results in a specific increase in the relative amounts of tRNA^{Tyr}-like material possibly by causing termination outside the tRNA genes. Though the presence of ρ considerably reduced the size of λ h80T RNA it seems to have only a minimal effect on the size of tRNA-like material. The distribution of the total tRNA-like material transcribed on λ h80T DNA (tRNA^{Gly}, tRNA^{Tyr} and tRNA^{Thr}) in the gradient fractions was determined as described in Fig. 2a by competition with the hybridisation of *E. coli* ³²P-tRNA to λ h80T DNA and was found to be similar to that observed for tRNA^{Tyr} chains (data not shown). In order to determine more accurately the size of the *in vitro* transcribed tRNA molecules we have analysed fractions 2 and 3 from the sucrose gradient by polyacrylamide gel electrophoresis in the presence of molecular size markers (Fig. 4). From the distribution of the radioactivity of RNA in the polyacrylamide gel, average molecular weights of 2.3×10^5 and 1.2×10^5 are observed for sucrose gradient fractions 2 and 3 respectively.

Are the three tRNA genes of λ h80T DNA part of a single transcriptional unit? Evidence bearing on this question may be obtained by considering the transcription of the tRNA₂^{Tyr} gene. This gene was shown, by electron microscopy studies by Wu *et al.*⁸, to be located between tRNA₂^{Gly} (su⁺₃₆) and tRNA₃^{Thr} genes at a distance of 140 and 260 nucleotides respectively. If the tRNA₂^{Tyr} gene is transcribed in a continuous sequence together with the adjacent tRNAs and spacer regions, the transcript should possess a much higher molecular weight than that of a mature tRNA. The purified RNA polymerase and factors used in *in vitro* studies is a suitable system for the detection of such primary transcription products since it lacks the precursor tRNA processing and modifying enzymes.

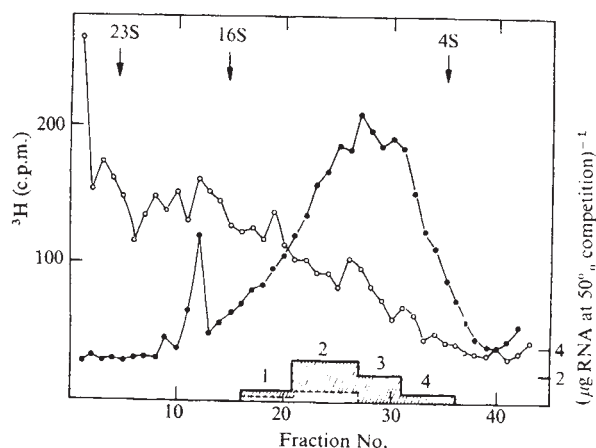


Fig. 3 Sucrose density gradient analysis of *in vitro* synthesised λ h80T RNA. λ h80T DNA (250 μ g) was transcribed *in vitro* by RNA polymerase holoenzyme in the presence (●—●) and absence (○—○) of ρ factor in reaction mixtures as described in legend to Fig. 2a. Each preparation of synthesised RNA was dissolved in 150 μ l of 1 $\times$ SSC, layered onto a 5 ml of 5–20% sucrose gradient in 1 $\times$ SSC and centrifuged for 4½ h at 50,000 r.p.m. and 4°C in SW 50.1 rotor Spinco model L centrifuge. Fractions of 7 drops were collected, aliquots of 1 μ l were precipitated with trichloroacetic acid, filtered through nitrocellulose filters and the radioactivity retained on the filters determined. In the same run, in a separate sucrose gradient, 23S, 16S, and 4S *E. coli* RNA were used as sedimentation constant markers. The histograms (ordinate at right) represent the tRNA₂^{Tyr} content of pooled fractions of RNA synthesised in the presence (|||||) and in the absence (—) of ρ factor. The tRNA₂^{Tyr} content was determined by competition with ³²P-tRNA hybridisation on ϕ 80psu⁺ L strand DNA as described in the legend for Fig. 2b. The relative amounts of tRNA^{Tyr}-like material are presented as the reciprocal of the *in vitro* synthesised RNA quantities which reduce by 50% the ³²P-tRNA hybridisation to ϕ 80psu⁺ L strand DNA.

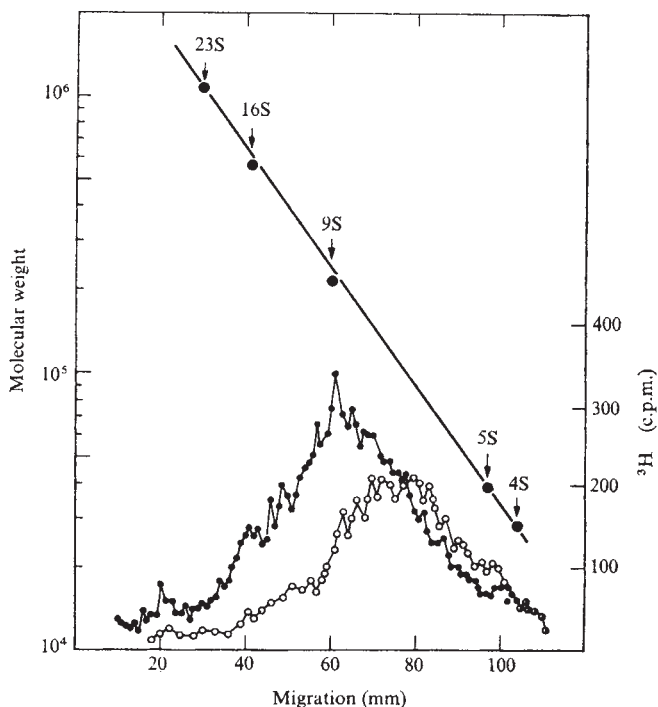


Fig. 4 Evaluation of the size of *in vitro* synthesised tRNA-like material by polyacrylamide gel electrophoresis. Fractions 2 (●—●) and 3 (○—○) of the λ h80T RNA synthesised *in vitro* by RNA polymerase in the presence of ρ factor (Fig. 3) were concentrated to 60 μ l and layered on to a 2% acrylamide, 0.5% agarose composite gel in Tris-EDTA-borate buffer (pH 8.3)¹⁹. 23S and 16S *E. coli* rRNA, 9S rabbit globin mRNA, 5S RNA and 4S tRNA were used as markers. The electrophoresis was run for 2 h at 0°C and 350 V. At the end of the run the markers were located by staining the gel with 'Stains all' (Eastman Kodak) and the distribution of the ³H-RNA determined by counting 1 mm slices dissolved in 0.3 ml NCS-tissue solubiliser and 3 ml toluene scintillator solution.

The tRNA₂^{Tyr} sequences transcribed *in vitro* were found mainly in RNA molecules with an average molecular weight of 230,000, a value close to that expected for a transcript of three tRNA genes of 80 nucleotides each and spacer regions of 140 and 260 nucleotides. Our findings thus favour the hypothesis of a polycistronic precursor⁵.

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JACOB I. GRIMBERG
VIOLET DANIEL

Department of Biochemistry,
The Weizmann Institute of Science,
Rehovot, Israel

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Uptake of *Escherichia coli* DNA into HeLa cells enhanced by amphotericin B

MAMMALIAN cells in culture take up small amounts of DNA, presumably by pinocytosis^{1,2}. Unlike bacterial cells the mammalian cells do not seem to require a special state of competence for DNA uptake³; and since naked DNA of oncogenic viruses can successfully infect or transform cells⁴, at least some of the DNA taken up by the cells can be functional. In spite of these successful experiments, however, almost all attempts to achieve controlled DNA transformation in mammalian cells have failed. The failure has been attributed to two factors: (1) insufficient transport of added DNA by the pinocytic mechanism¹; and (2) degradation of the foreign DNA in lysosomes⁵.

Modifications of the input DNA by % irradiation or shear⁶⁻¹³ or partial degradation by nucleases¹⁴ have generally only reduced uptake. A few complex polycations, including DEAE-dextran¹⁵, protamine sulphate¹⁶, polylysine and polyornithine¹⁷, do enhance transport into cultured human cells. The enhancement is however at the most two to three-fold. Also the treatments and effects of these agents are difficult to reverse or control, and some of the agents form insoluble complexes with DNA^{18,19} that may not be easily assimilated by cells.

Encouraged by its capacity to promote uptake of a variety of antibiotics into fungal²⁰ and animal²¹ cells, we have tested the polyene macrolide antibiotic amphotericin B (AmB) as a potential agent to enhance uptake of DNA into HeLa cells. Here we report that while the levels of AmB required are high, the transport of DNA is increased 8 to 10-fold under optimal conditions without affecting subsequent cell viability or growth. The process is reversible and can be controlled, because the increased transport stops when the cells are washed free of AmB. Furthermore, the DNA taken up in the presence of AmB is less altered than that transported in its absence.

Figure 1 shows the kinetics of transport of *E. coli* DNA into HeLa cells in culture in the presence of different concentrations of AmB. Acid-precipitable radioactivity was measured in cells after they were washed with medium containing sodium iodoacetate to remove any nonspecifically bound DNA²². Samples incubated without AmB showed the low level of transport already reported²³, whereas samples that contained more than 20 $\mu\text{g ml}^{-1}$ of AmB showed a considerable enhancement in the transport of DNA. The transport was linear for 30 min, and reached a plateau value at 30 to 60 min. In some experiments, further incubation caused a loss of 10%-30% of the net internalised DNA, an observation similar to some earlier reports^{23,24}. After 3 h of incubation at the concentrations of AmB shown, 85-90% of the cells were still viable, as judged by trypan blue dye exclusion. Washed free of DNA and AmB, the

cells uniformly resumed growth and 24 h later had grown as well as untreated cells.

The interdependence of cell number, DNA concentration, and AmB concentration in determining the level of DNA uptake observed is comparable with that observed in the potentiation of antibiotic uptake into fungal cells by AmB (E. Battaner and B. V. K., unpublished work). Figure 2 shows this interdependence, where the ratio of drug to cell number is shown to be particularly important. This

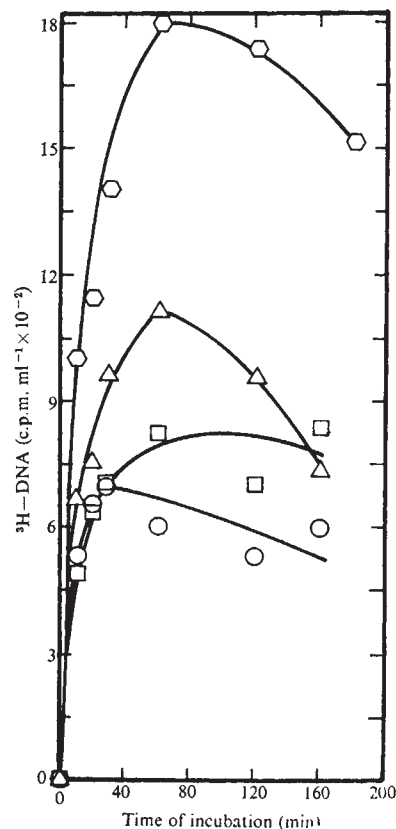


Fig. 1 Kinetics of transport of DNA by HeLa cells. Cells grown in spinner culture in Joklick modified Eagle's medium supplemented with 5% horse serum were centrifuged and suspended in the same medium, but with 10% calf serum. The dispersed cells were counted in a haemocytometer, and then dispensed in 4.5 ml portions into flasks at 37°C with a culture of strain 15T⁻, a thymine auxotroph with ³H-thymine for several generations. DNA was extracted according to ref. 28. It had a specific activity of 12,000 c.p.m. μg^{-1} and showed a characteristic narrow sedimentation distribution in a sucrose gradient at 20°C. At specified time intervals portions of cell suspension were centrifuged and washed twice in incubation medium containing 0.025 M sodium iodoacetate in the cold at pH 7.3 (ref. 22). Each equivalent of 0.5 ml of cell suspension was precipitated with 5% CCl_3COOH . The precipitate was collected on a glass fibre filter and counted in a toluene-based scintillant in a Packard Spectrometer. Acid precipitable radioactivity was plotted per ml of cell suspension. Amphotericin B concentrations used were 200 ($\bigcirc$), 100 ($\bigcirc$) and 20 ($\square$) μg per ml of cell suspension.

is probably because of the large number of sterol binding sites for AmB per cell²⁵. For example, in the conditions of Fig. 1 with 2.3×10^5 cells ml^{-1} , there was 3.3 times more DNA uptake in the presence of 200 $\mu\text{g ml}^{-1}$ AmB. In optimal conditions with 4.5×10^5 cells ml^{-1} as in Fig. 2, there was 8 to 10 times more DNA uptake.

Transport was inhibited 85-90% by 10 mM sodium cyanide (Fig. 2) which is consistent with the idea that

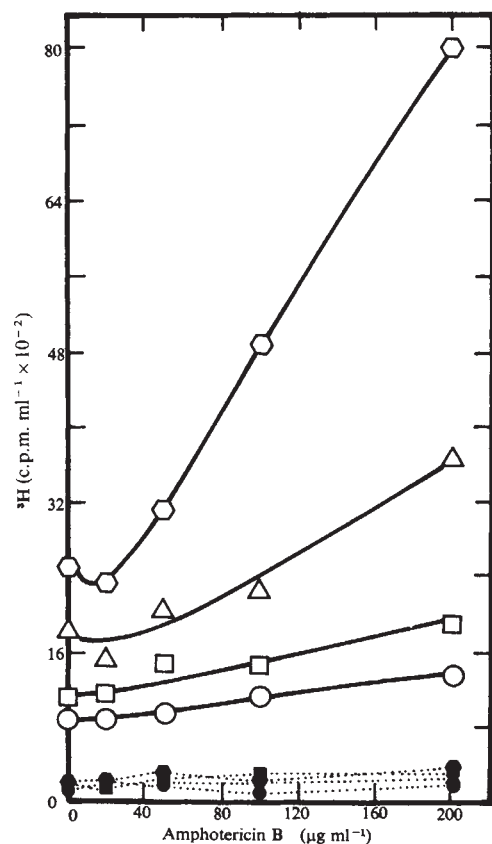


Fig. 2 Transport of DNA by HeLa cells. HeLa cells (4.5×10^6 cell ml^{-1}) were resuspended as in Fig. 1 and incubated in 0.5 ml portions at 37°C with specified concentrations of amphotericin B and *E. coli* ^3H -DNA. After 30 min, cells were washed and acid-precipitable radioactivity was measured as in Fig. 1. The response to AmB and DNA concentration is indicated. 4.5×10^5 cells ml^{-1} incubated with 2 (○), 4 (□), 8 (△) and 20 (◇) μg of ^3H -DNA for 30 min at 37°C at the indicated concentrations of amphotericin B. A second set of samples incubated under identical conditions contained 10 mM sodium cyanide with 2 (●), 4 (■), 8 (▲) and 20 (◆) μg of ^3H -DNA ml^{-1} .

uptake might depend on an active process such as pinocytosis²⁶. Also, uptake was temperature dependent: at 0°C , it was completely inhibited in the absence of AmB, and was inhibited 50–60% in its presence.

The effect of AmB on DNA uptake seems to require its simultaneous presence in the medium. For example, when cells were pretreated with AmB (Fig. 3) and washed free of AmB before incubation with DNA, they took up DNA only to the same extent as cells that had not been treated with AmB. This suggests that the binding of the drug to the cell membrane is reversible or temporary in its effect, and confirms that the short time of exposure of the cells to high concentrations of AmB is nontoxic and reversible.

When HeLa cells were incubated with different concentrations of AmB and *E. coli* DNA for 30 min at 37°C , then washed free of AmB and DNA and incubated further in fresh medium, it was found that the acid-precipitable radioactivity in the cells declined steadily. After 8 h only about two thirds of the DNA taken up in untreated cells, and one-third of that transported in presence of AmB was still recoverable from cells. But the DNA extracted from the AmB treated cultures at any one time was relatively more intact, as judged by the sedimentation behaviour of the two DNA samples on an alkaline sucrose density gradient (data not shown).

The possibility that bacterial DNA might be preserved intact longer in AmB treated cells was further supported by DNA-DNA hybridisation trials (Fig. 4). Labelled DNA re-extracted from cells treated with $200 \mu\text{g}$ of AmB ml^{-1} for 30 min reannealed to an extent indistinguishable from the input bacterial DNA; at this time, 70% of the label in cell incubated without AmB hybridised much more slowly. Very probably, it had already been degraded and the products reincorporated at random into HeLa cell sequences.

The apparently prolonged intact survival of DNA sequences taken up in the presence of AmB could increase the probability of it functioning in the recipient cell. The binding of AmB to membrane sterols may increase endocytosis in a way that avoids a direct route to lysosomal vacuoles; or alternatively, the number of new phagosomes simply saturate the lysosomal complement of the cell for some time. At any rate, the advantage of increased survival of intact DNA, combined with the increased levels of uptake and the easy reversibility of the AmB effect, makes AmB a promising agent for permeability control.

Though it is true that our arguments for the incorporation of the DNA into the cells are indirect, two kinds of data support the inference that AmB truly promotes uptake rather than a loose ionic association of DNA with the cell membrane. First, these concentrations of AmB potentiate uptake of a variety of antibiotics^{20,21,27} and of macromolecules that include RNase, DNase and even latex beads $1.1 \mu\text{m}$ in diameter (to be published). Second, the results satisfy the criteria for uptake in previous studies with DNA, including the requirement for energy and the resistance to extensive washing with iodoacetate. We are now testing

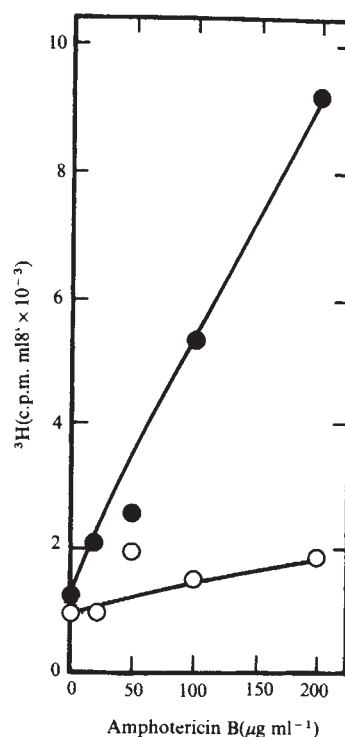


Fig. 3 Effect of pretreatment of cells with amphotericin B on the uptake of DNA. HeLa cells (5.0×10^5 cells ml^{-1}) were washed as in Fig. 1 with medium containing sodium iodoacetate and incubated at 37°C for 30 min in 0.5 ml fractions with specified concentrations of amphotericin B. After incubation, cells were washed free of amphotericin B and resuspended in the same volume with $4.4 \mu\text{g}$ of ^3H -DNA (specific activity $24,500$ c.p.m. μg^{-1}). Incubation was then resumed in the presence (●) or absence (○) of amphotericin B at the indicated levels. Cells were then washed and assayed for incorporated DNA as in Fig. 1.

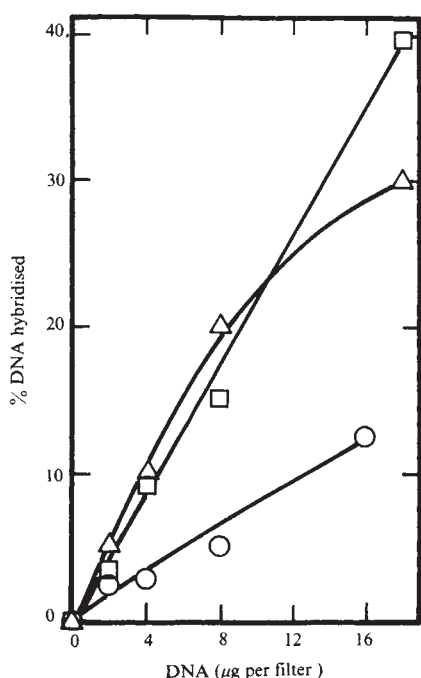


Fig. 4 Reannealing of DNA reisolated from HeLa cells which had taken up *E. coli* ³H-DNA. HeLa cells (2.5×10^5 cells ml^{-1}) were incubated with $200 \mu\text{g ml}^{-1}$ (Δ) or $0 \mu\text{g}$ ($\circ$) of amphotericin B in the presence of $45 \mu\text{g}$ of *E. coli* ³H-DNA (specific activity $18,400 \text{ c.p.m. } \mu\text{g}^{-1}$) at 37°C for 30 min. After incubation, cells (15×10^6) were washed and DNA was re-extracted²⁸. DNA was then heat denatured to single strands and DNA-DNA hybridisation was conducted essentially according to ref. 29. In a control experiment a comparable amount of *E. coli* ³H-DNA that had not been exposed to HeLa cells was denatured and its capacity to reanneal with itself was measured ($\square$). The reaction mixtures each contained $8 \mu\text{g}$ ($1,030 \text{ c.p.m.}$) of labelled DNA and the specified amount of unlabelled *E. coli* DNA. Another set of control samples was incubated with HeLa cell DNA and a blank filter. The low levels of radioactivity nonspecifically absorbed to the control filters (about 5% of the experimental values) were subtracted from the test samples before calculating the percentage hybridisation.

for the expression of the DNA incorporated into the cells.

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B. VIJAYA KUMAR
GERALD MEDOFF
GEORGE KOBAYASHI
DAVID SCHLESSINGER

Departments of Microbiology and Medicine,
Divisions of Infectious Diseases and Dermatology,
Washington University School of Medicine,
St. Louis, Missouri 63110

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Effect of a protein-free diet on mitotic activity of transplanted splenic lymphocytes

THE graft *versus* host reaction (GvHR) is initiated by donor thymus-derived lymphocytes¹⁻³, and, the defence reaction of the host seems to be influenced by the extent of host lymphoid tissue^{4,5}. Dietary protein deprivation provokes an involution of the lymphoid organs, mainly of the thymus and of the thymus-dependent fraction of blood and lymphoid tissue lymphocytes^{6,7}. It is therefore of interest to know if a protein-free diet given to donors of a spleen graft inhibits the GvHR reactivity of the grafted lymphocytes, and if the same diet given to recipients lowers the capacity of these animals to react against the grafted cells.

The latter hypothesis has already been confirmed⁸. A local lymph node GvHR, produced in hybrid F₁ rats by injection of parental spleen lymphocytes, was reported to be much weaker in protein-deprived recipients than in normally fed ones. In contrast, the weight response of the popliteal lymph node after subplantar injection of lymphocytes from protein-deprived parental rats was not reduced. Here, I report the influence of protein deprivation of donors or recipients on the proliferation of grafted lymphocytes recognised by the Y chromosome.

Adult female (Sherman $\times$ Wistar) F₁ hybrid rats were inoculated intraperitoneally with splenic lymphocytes from male parental (Sherman) rats. The cell suspensions were prepared as described previously⁸. The dose of injected viable cells (unstained by erythrosine) was 120×10^6 or 60×10^6 depending on whether the recipients were fed normally or had been fed on a protein-free diet for the previous two months. (For composition of diet see ref. 9.) The terminal body weight was about 210 g for the normal rats and 105 g for the deficient ones.

The donors also included normally fed and protein-deprived rats. Among the former, 12 rats had been thymectomised at 25 d of age. These were introduced into the study to see whether the thymic atrophy of protein-deprived donors could be responsible for the blocking of the multiplication of grafted cells derived from these rats.

Table 1 Mitoses of donor cells in spleen of recipient rats during first week of a graft *versus* host reaction

Strain and sex		Diet		6 h		Time since transplantation		7 d	
Donor	Recipient	Donor	Recipient	Total mitoses %	Donor mitoses %	Total mitoses %	Donor mitoses %	Total mitoses %	Donor mitoses %
Sherman	(Sherman × Wistar) F ₁	Normal	Normal	7.43 ±0.75 (7)	19.93 ±7.05 (7)	13.67 ±0.92 (6)	14.51 ±2.08 (5)	18.20 ±1.91 (5)	6.93 ±1.99 (5)
♂	♀	Normal	Protein-free	1.71 ±0.03 (7)	0	7.50 ±0.99 (6)	11.00 ±4.30 (6)	8.67 ±1.98 (6)	17.67 ±3.80 (6)
♂	♀	Protein-free	Normal	6.00 ±0.38 (7)	12.23 ±3.73 (7)	15.50 ±1.77 (6)	0	19.33 ±2.73 (6)	0.93 ±0.93 (6)
♂	♀	Protein-free	Protein-free	—	—	7.17 ±0.70 (6)	6.36 ±0.94 (6)	11.40 ±3.04 (6)	21.00 ±3.67 (6)
Thymect. Sherman ♂	♀	Normal	Normal	4.71 ±0.36 (7)	0.79 ±0.79 (7)	9.00 ±1.61 (5)	9.16 ±5.67 (5)	—	—
Sherman ♂	Sherman ♀ (Syngeneic controls)	Normal	Normal	—	—	9.60 ±0.93 (5)	2.00 ±1.23 (5)	4.75 ±0.85 (5)	—
Uninjected Sherman ♀		Normal		—	—	4.00 ±0.63 (5)	—	—	—

Values are means ± s.e. The numbers of rats are indicated in parentheses. All recipient rats had been injected intraperitoneally 1 h 30 min before killing with 0.25 mg per 100 g of colchicine. The total number of mitoses was established on chromosomal preparations performed according to ref. 13 and stained with Unna's Blue. The percentages of mitoses of the male donor cells were counted after construction of karyotypes from 100 selected mitotic figures.

The F₁ recipients were killed 6 h, 48 h or 7 d after the transplantation. Uninjected female Sherman controls or female Sherman rats injected with 120×10^6 male Sherman lymphocytes and killed after 48 h or 7 d were also studied to exclude GvHR-independent mitotic activity.

As shown in Table 1, normally fed F₁ recipients inoculated with normal parental lymphocytes exhibited a progressive increase in the mitotic index between days 1 and 8, whereas the percentage of male (donor) mitoses dropped from about 20 to 7. These results agree with already published reports¹⁰⁻¹² indicating that cellular proliferation in the spleen, induced by GvHR, is mainly of host origin. In normal recipients inoculated with lymphocytes from protein-deprived donors, the total number of mitoses per 10^3 cells showed the same changes as in the above group but the mitoses of the grafted cells disappeared almost completely after 48 h and 7 d. As shown by the total number of mitotic figures, the host reaction was at least as important as after inoculation of normal parental lymphocytes.

If the protein-free diet was given not to the donors but to the recipients, splenic mitoses were much less numerous than in normal recipients although their number increased between days 1 and 8. No donor cell mitoses were detected 6 h after transplantation but the initial inhibition of the grafted cells was only temporary and after 7 d the number of male mitoses became even significantly higher in protein-deprived than in normal recipients. Since the proliferation of the parental lymphocytes seems to be conditioned by continuous stimulation exercised by alloantigens of the host cells¹², it seems that the latter cells preserve their capacity of stimulating the donor lymphocytes, although (partly) losing their ability to defend themselves against their aggressors.

If both donors and recipients were deprived of proteins, the total number of mitotic figures was as low as in the preceding group, although higher than in uninjected rats. But the multiplication of donor cells from protein-deprived parents was not at all impaired after contact with cells of protein-deprived recipients while it was, as seen above, inhibited by lymphocytes of normally fed F₁ hybrids.

The inability of lymphocytes from protein-deprived donors to divide in the spleen of normal recipients could not be attributed exclusively to the low content of the graft in GvH-reactive T

lymphocytes because of the thymic atrophy of the donors. Indeed, if the transplanted lymphocytes came from normally fed but thymectomised parental rats, the multiplication of these cells in the recipients, although completely inhibited in all rats during the first hours and in some rats even 2 d after inoculation, reached normal levels in other rats after 2 d. Since thymic atrophy cannot be more harmful than late thymectomy, it may well be that the suppression of mitotic division of cells from protein-deprived donors may be due to inactivation of donor B lymphocytes as well as to inactivation of T lymphocytes.

Assuming that the GvHR-initiating role of the donor T cells consists in the recognition of foreign histocompatibility antigens of the host¹², and that this recognition is followed by production of antibodies by donor B lymphocytes against these antigens, it may be that protein deprivation of donor rats does not completely impair the capacity of their T lymphocytes to recognise alloantigens but that the immunological response of their B cells is prevented. This naturally facilitates the host reaction against the grafted lymphocytes, from protein-deprived donors, which may explain the blocking of proliferation of the latter cells.

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A. ASCHKENASY

Centre National de la Recherche Scientifique,
Centre Marcel Delépine,
Laboratoire d'Hématologie nutritionnelle,
45045 Orléans, France

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Neutral evolution and immunoglobulin diversity

THE origin of immunoglobulin diversity and species specificity continues to be a controversial topic. Proponents of the germ line theory have presented a limited gene expansion-contraction model in general terms¹⁻³; the mechanism has not yet been demonstrated to produce sufficiently rapid changes in a multi-gene system to account for the observed phenomena. Species divergence of immunoglobulin *V* regions continues to be cited as strong evidence against multiple germ line *V* genes^{4,5}. We report here computer simulation of random unequal crossing over within a system of multiple related genes and compare the results with the relationships observed between immunoglobulin *V* region sequences from different species. We conclude that the species-specific features of immunoglobulin *V* regions can arise as a result of such random processes operating in the multigene set.

We assume that the previous evolutionary history of the organism established a set of genes by repeated duplication and accumulation of point mutations such that the individual genes are homologous but not necessarily identical. The homology is compatible with continued unequal crossing over. This event and its consequences are illustrated in Fig. 1. In the absence of any meiotic distortion the probability of the progeny receiving a chromosome with one additional gene or with one less gene is equal. In the absence of selective pressures, the new duplication or deletion, in itself, would represent a neutral mutation and as such would become fixed in the population at a rate equal to the rate of occurrence of the event⁶.

We simulated the evolution of 50 related genes by unequal crossing over in the absence of selection. At each step of an iterative process, a gene occupying one of the positions in the linear array was selected on a random basis and was either duplicated in the array or deleted. At each event, only the duplicated or the deleted product was chosen at random and was then assumed to become fixed in the population as a neutral mutation before the next repetition. Figure 2 is the result of 200 repetitive cycles. Comparison of the evolved sequence with the initial sequence shows that some of the original genes have been eliminated and replaced by duplications of other original genes. The consequence is evolution of subgroups of closely related genes within a set of related genes. Point mutations and single codon additions or deletions will occur within genes of a subgroup during its evolutionary history. These events and the structure of the ancestral gene of each subgroup will give the characteristic features of the subgroup and provide variation within the individual members.

Speciation divides a common gene pool and permits random mutational events to act on the two resulting pools in an independent manner. This was simulated by taking the original set of 50 related genes and repeating the 200 cycles of unequal cross overs under separate random control. Figure 3 compares the results of independent evolution of the two gene pools. In most cases, the remaining genes in the second example differ from those in the first as do the subgroups and their distribution. Speciation and subsequent independent evolution by this mechanism in the absence of any selective forces does permit multiple homologous but nonidentical genes to evolve and produce species-specific subgroups which have descended from different ancestral genes.

The simulation has been presented in terms of 50 genes;

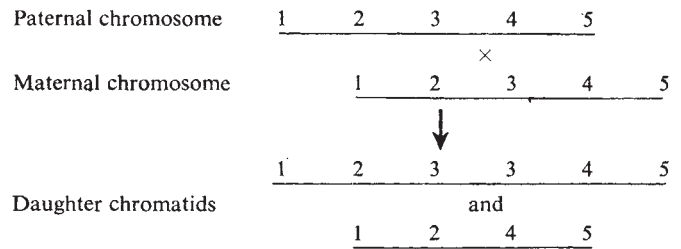


Fig. 1 Unequal crossing over between tandem sets of homologous genes and the products of the event.

however, the results are valid for larger sets, as shown in Fig. 4. Within the limits of the random process, the number of cross overs required for any given reduction in number of subgroups is directly proportional to the number of original genes. For example, 250 genes are reduced by 60% after 600 cross overs while 500 genes are reduced by 60% after 1,200 cross overs. Fifty genes are reduced to 1 subgroup after 2,000 cross overs and by extrapolation 1,000 genes would be reduced to 20 subgroups after 40,000 cross overs.

The number of subgroups present in any species will depend on the number of unequal cross overs which have been established following speciation (Fig. 4) and will also vary due to the random nature of the events involved. The model anticipates different numbers of subgroups in different species, such as appear to exist in mouse and human kappa chains^{1,7}. Although the total number of *V* genes tends to remain at a constant average level, random fluctuations about this mean value occur during any one simulation. The model could thus account for the differences in the proportion of kappa to lambda chains observed in different species. Similarly, variations in the proportions of the heavy chain subgroups in different species are expected⁴.

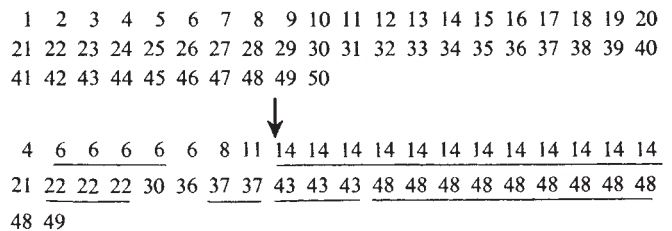


Fig. 2 The origin of subgroups by unequal crossing over. The top set of numbers represent 50 homologous but nonidentical genes arranged in tandem order. Gene deletion and duplication was simulated as shown in Fig. 1 with random selection of the gene involved and of the deleted or duplicated product. After 200 unequal cross overs the bottom set of numbers was obtained.

The model as presented in its simplest form can account for many of the documented facts concerning the evolution of *V* region sequences⁷. The model depends on cross overs which are ubiquitous genetic events⁸. Once the initial unequal cross over has taken place in the multiple gene system, subsequent recombinations involving either the deleted or duplicated product will also be unequal and the process will be self propagating⁹. The frequency of unequal crossing over could approach the frequency of recombination which has been estimated to be 2×10^{-3} for two markers separated by 1,000 tandem *V* genes¹. If the average mammalian generation time is 4 yr¹⁰, one neutral unequal cross over would be established every 2,000 yr where the rate of fixation is equal to the rate of occurrence⁶. If the kappa *V* gene pool contains 1,000 genes, the data in Fig. 4 suggest that 40,000 cross overs would be required to establish the present species divergence between mouse and human kappa

4	6	6	6	6	6	8	11	14	14	14	14	14	14	14	14	14	14	14	14
21	22	22	22	30	36	37	37	43	43	43	48	48	48	48	48	48	48	48	48
48	49																		
3	7	7	12	15	15	15	15	18	18	18	19	31	41	41	41	41	41	41	41
41	41	41	41	41	41	41	41	41	41	41	42	42	42	42	42	42	42	43	43
50	50	50	50	50	50	50	50												

Fig. 3 The effect of speciation on evolution by unequal crossing over. The top set of numbers are the product of 200 simulated cross overs as shown in Fig. 2. Speciation was simulated by taking the original set of 50 numbers and repeating the 200 unequal cross overs under separate random control. The bottom set of numbers is the result of the second series of unrelated random processes.

chains. This would take 8×10^7 yr which is the time estimated for evolution of the primates¹¹. The model is therefore compatible with a pool size of 1,000 genes and the evolutionary time scale.

Although the model has been tested in the absence of any selective pressures, it is obvious that these must exist. Simple determinants elicit production of several variable region sequences which suggests that considerable redundancy or degeneracy exists in the immune system¹²⁻¹³. A single V region sequence with the unique capacity to combine with a given pathogenic determinant⁷ is probably rare. We feel that the major role of selection will be to prevent deletion of the final copy of certain genes essential for immunological survival of the species in its indigenous pathogenic environment. Deletion of the corresponding gene would be lethal. Simulation of this situation gives a result which is not significantly different from the simulation without selection.

The model does not require extensive reduction or increase in gene number within the multiple gene system during evolution. Therefore, a fully functional immunological system will be available to the evolving species at all times. The past immunological experience of the species will be contained in the gene copies and may be subject to gradual elimination only if these genes are of no selective advantage to the present phenotype. The model permits a response to present immunological challenges based on past experience while maintaining adequate options to meet future attacks.

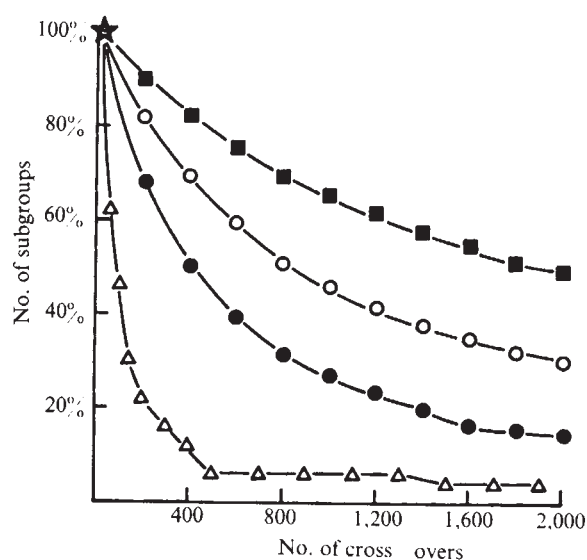


Fig. 4 The effect of the number of unequal cross overs and size of the initial gene set on the number of subgroups. The number of subgroups are expressed as a percentage of the original number of genes in the set. Δ , 50 initial genes; $\bullet$, 250 initial genes; $\circ$, 500 initial genes; $\blacksquare$, 1,000 initial genes. The star represents the convergence of all four curves at 100%.

Note added in proof. Smith (Cold Spring Harbor Symp., in the press) has also used computer simulation to reach conclusions similar to those presented here.

JOHN A. BLACK

Department of Biochemistry and Division of Medical Genetics,
University of Oregon Medical School,
Portland, Oregon 97201

DAVID GIBSON

Department of Biochemistry,
University of Sherbrooke,
Sherbrooke, Quebec, Canada

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Surface antigen(s) common to human astrocytoma cells

SINCE animal tumours induced by viruses exhibit common antigenic properties¹, the extent to which antigens are shared in human tumours is of obvious interest. Such antigenic cross reactions between human tumours of the same histological origin have been demonstrated by cell-mediated colony inhibition testing as well as by serological means^{2,3}. But few immunological studies of human brain tumours have been reported. Studying the cytotoxic effect of patients' lymphocytes on autologous or allogeneic astrocytomas, some workers have found antigenic cross reaction⁴ while others have not⁵. I report here the serological demonstration of surface antigen(s) common to cultured human astrocytoma cells.

Surgically removed human cerebral tumours were mechanically disaggregated and maintained in HEPES-buffered Eagle's Minimum Essential Medium with Earle's salts, 10% horse serum, 5% foetal bovine serum, glucose (3 mg ml⁻¹), glutamine (0.3 mg ml⁻¹), penicillin (50 units ml⁻¹), streptomycin (50 µg ml⁻¹) and Fungizone (2.5 µg ml⁻¹) (M. S. Lakshmi, personal communication.) The cells used for immunisation were those designated Astrocytoma 301, cultures of which were shown to be mycoplasma- and virus-free and of glial morphology by electron microscopy. The cells were collected in EDTA (0.02%) and washed in phosphate-buffered saline (PBS), pH 7.2. A New Zealand white rabbit was injected intravenously with 25×10^6 cells on days 0 and 14, and bled out on day 24. The serum was heated for 30 min at 56° C and its antibody activity assayed by dye exclusion cytotoxicity testing⁶ using cultured astrocytoma cells as targets and guinea pig serum as the source of complement. The antiserum was repeatedly absorbed with normal brain homogenate until further absorptions did not decrease the cytotoxicity against Astrocytoma 301 cells. This required four absorptions (Fig. 1a).

Brain homogenate was no more effective in decreasing cytotoxic activity than liver, spleen, kidney, myocardium or thyroid homogenates (Fig. 1a, b). On a volume per volume basis, brain was no more efficient in absorbing the original

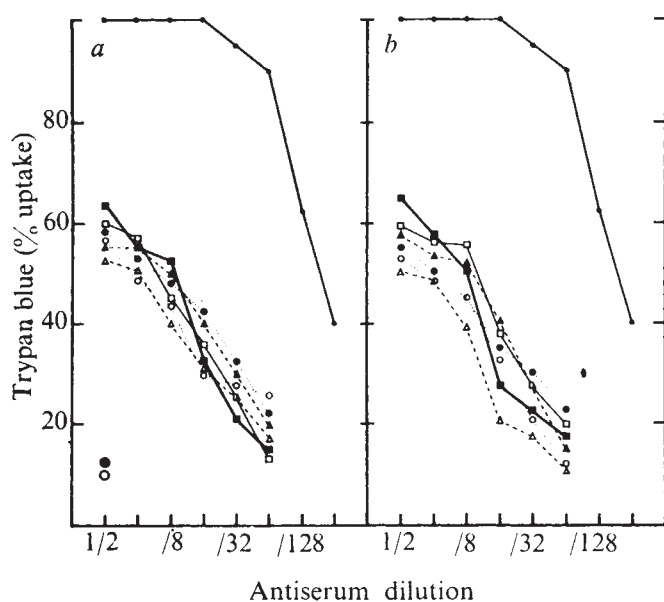


Fig. 1 Dye exclusion cytotoxicity tests showing the activity of unabsorbed anti-astrocytoma 301 serum (●—●) and of the antiserum absorbed *a*, $\times 4$ or *b*, $\times 6$ with human brain (■—■), liver (●—●), spleen (○—○), kidney (□—□), heart (△—△) and thyroid (▲—▲). Astrocytoma 301 cells, between third and sixth passage were collected in EDTA (0.02%), washed and suspended in phosphate-buffered saline (pH 7.2) with 0.1% bovine serum albumin (PBS) at 10^6 cells ml^{-1} . 25 μl of cells were incubated with 25 μl of doubling dilutions of antiserum for 30 min at 37° C; after washing once, cells were resuspended in fresh unabsorbed guinea pig serum diluted 1:4 in PBS for 30 min at 37° C following which the number of dead cells was assessed using trypan blue staining. For absorption, tissues were homogenised in a Potter-Elvehjem homogeniser, and washed $\times 3$ in PBS. The antiserum was mixed with equal volume of packed homogenate (centrifuged $3,600g \times 10$ min) and absorbed for 30 min at 20° C with continuous shaking. ●, Normal serum; ○, complement.

Table 1 Absorption of anti-HAAA by non-astrocytoma cultures and homogenates

Culture	Passage no.	Absorbing material	Origin	% kill* of anti-HAAA			
				5	2.5	1.25	0.62
212	8	Meningioma		41	42	42	40
217	4	Meningioma		41	42	43	44
222	3	Meningioma		40	43	42	45
U2/18†	18	Breast epithelioma		46	45	43	43
U1/18†	18	Breast carcinoma		41	52	50	42
U3/15†	15	Breast carcinoma		43	43	44	42
U7/2†	2	Breast fibroadenoma		37	40	45	48
U9/16†	16	Breast fibroadenoma		39	40	45	47
MRC 5†	—	Human diploid fibroblasts		40	42	45	47
HeLa†	—	Carcinoma cervix		43	43	46	46
HFB.22	7	Foetal brain (22 week stage)		44	42	43	44
HFB.22		Foetal brain§ (homogenate)		50	49	46	46
HFB.12		Foetal brain§ (12 week stage)		48	47	47	45
Brain absorbed anti-HAAA serum				53	52	52	50

*25 μl anti-HAAA diluted 1:12, absorbed at 37° C as in Fig. 2 with doubling dilutions of cells ($\times 10^6$). Kill with normal rabbit serum was $< 10\%$.

† Cells donated by Dr P. A. Riley.

‡ Established line.

§ Doubling dilutions of 25 μl packed homogenate were used. Controls were used as in Figs 1 and 2.

antiserum than other tissues tested; it is therefore unlikely that there were any brain-specific antibodies in the original serum. Since the absorbed antiserum could not be further absorbed by a number of nonastrocytoma cells, but could be completely absorbed with cultured cells from each of seven different astrocytomas (Fig. 2, Table 1), I shall refer to the antigen(s) defined by it as human astrocytoma-associated antigen(s) (HAAA). When target cells were collected mechanically, by treatment with EDTA (0.02%) or trypsin (0.02%), similar results were obtained. Absorption of anti-HAAA activity by homogenates of two original astrocytoma (301 and A3) samples, stored at -165°C , indicates the presence of this antigen *in vivo*.

In view of the failure of cultured cells from foetal brain or normal brain homogenates to absorb the anti-HAAA activity (Fig. 2, Table 1) it seems unlikely that HAAA is an

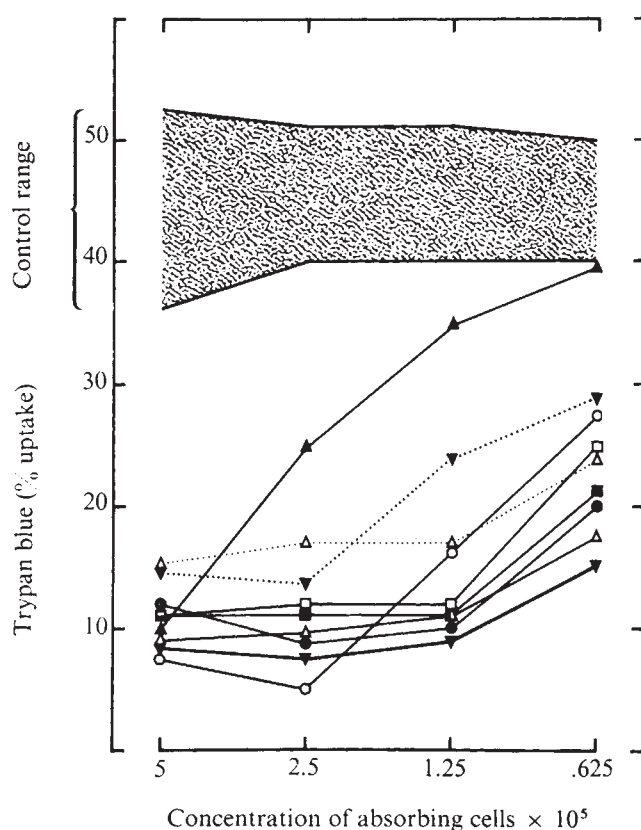


Fig. 2 Dye exclusion cytotoxicity tests showing the ability of cultured cells from seven different astrocytomas, A1 (●—●), A2 (○—○), A3 (▲—▲), A4 (△—△), A5 (■—■), A6 (□—□), and 301 (▼—▼) and homogenates of 301 (▼—▼) and A3 (▲—▲) to absorb anti-HAAA activity. Results of absorptions with various non-astrocytoma cells and homogenates fall within the cross-hatched area and are given in detail in Table 1. 25 μl of anti-HAAA diluted 1:12 in PBS was absorbed with doubling dilutions of a suspension of whole cells or homogenates (50% v/v) for 30 min at 37° C and the absorbed serum tested as in Fig. 1, with unabsorbed rabbit serum diluted 1:10 as complement.

oncofoetal antigen or an astrocyte differentiation antigen, although the former cannot be entirely excluded since HAAA expression could be restricted to a stage of embryogenesis not represented by the samples available here. It is also possible that absorption with normal adult brain tissue from more individuals would reveal that HAAA is normally expressed in a proportion of normal brains, making it an alloantigen analogous to the thymus-leukaemia (TL) antigen in mice⁷. The exact significance of the common antigen detected here remains in doubt but could suggest a common viral aetiology.

(n.t. = not tested).

activity that these compounds interrupt pregnancy at all stages in the rat.

The luteolytic potency of these agents is, however, in no way related to their capacity to stimulate smooth muscle. This was assessed *in vitro* from their potency in provoking isotonic contractions of the isolated uterus of dioestrous guinea-pigs and of segments of gerbil colon (Table 2).

As a smooth muscle stimulant, ICI 80,996 is similar in potency to $\text{PGF}_{2\alpha}$, whereas ICI 79,939 is 10 times, but ICI 81,008 only 1/50 as potent.

In general, the severity of the side effects of these compounds parallels their potency as stimulants of smooth muscle. With ICI 79,939, early pregnancy can be terminated in hamsters and even in rats at doses that have no obvious side effects: these doses, however, are toxic to nonpregnant animals and sometimes lethal to young ones. In the doses needed to interrupt pregnancy in these species, ICI 80,996 and ICI 81,008 seem completely devoid of side effects in both pregnant and nonpregnant animals. Moreover, large multiples ($\times 20$ or more) of these doses can be given without causing serious distress. With $\text{PGF}_{2\alpha}$, side effects are sometimes seen at doses below the minimum required to terminate pregnancy. When seen, the side effects of the analogues are similar to those of $\text{PGF}_{2\alpha}$ and include watery diarrhoea, hypersalivation, gasping, abdominal 'squirming', ataxia, reduced motility, irritability and weight loss.

Table 2 Relative potencies of $\text{PGF}_{2\alpha}$, its analogues and PGE_2 in stimulating contractions of smooth muscle *in vitro*

Compound	Guinea pig uterus	Gerbil colon
$\text{PGF}_{2\alpha}$	1 (assigned)	1 (assigned)
ICI 79,939	10	9.5
ICI 80,996	1	0.6
ICI 81,008	0.02	0.036
PGE_2	36	—

From our results it seems that, while the analogues described are similar in luteolytic potency and all are many times as potent as $\text{PGF}_{2\alpha}$, they differ markedly as smooth muscle stimulants and in toxicity. By the criteria described above, ICI 79,939 is more toxic than $\text{PGF}_{2\alpha}$, ICI 80,996 less toxic, and ICI 81,008 very much less toxic.

Reports have already appeared on the luteolytic activity of these analogues in farm animals^{4,5} and their potential for the control of oestrus and the treatment of infertility in cattle⁴ and horses^{5,6} and the induction of farrowing in pigs⁷. Results in pig-tail monkeys (*Macaca nemestrina*) suggest that ICI 80,996 and, more particularly, ICI 81,008 may also be of value as antifertility agents in women. A s.c. dose of $50 \mu\text{g kg}^{-1}$ of ICI 80,996 or $100 \mu\text{g kg}^{-1}$ of ICI 81,008 given on day 20 of the (30–31 d) cycle and on the next 4 to 5 d, causes menstrual bleeding 4 to 7 d before the expected time. The rapid fall in plasma progesterone during treatment and before the onset of bleeding indicates that this is due to luteolysis. Several of the monkeys given ICI 80,996 suffered transient side effects, chiefly diarrhoea, but with ICI 81,008 these were infrequent and mild.

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M. DUKES
W. RUSSELL
A. L. WALPOLE

ICI Ltd (Pharmaceuticals Division),
Mereside,
Alderley Park,
Macclesfield, Cheshire, UK

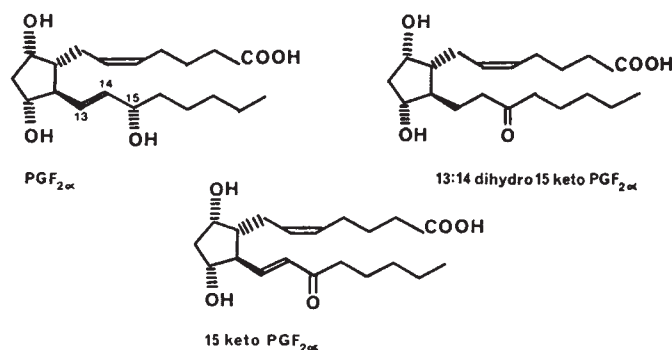
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Potent bronchoconstrictor activity of 15-keto prostaglandin $\text{F}_{2\alpha}$

THE effects of parent prostaglandins (PG) on bronchial muscle *in vitro*¹⁻³ and *in vivo*^{4,5} have been characterised, but the pharmacological properties of their metabolites are less well defined. The lung is an important site of PG metabolism and a better understanding of the effects of the various metabolites is necessary since PGs are now recognised as compounds released in asthma. Modification of PG metabolism itself could be responsible for the aetiology of the asthmatic bronchospasm, particularly since asthmatic subjects have been shown to be considerably more sensitive to inhaled $\text{PGF}_{2\alpha}$ than the normal population⁶. We have shown that 15-keto $\text{PGF}_{2\alpha}$ possesses very potent bronchoconstrictor activity and is probably important in asthma.

Two enzymes in the lung are responsible for metabolising PG: 15-hydroxy prostaglandin dehydrogenase, which converts, for example, $\text{PGF}_{2\alpha}$ to 15-keto $\text{PGF}_{2\alpha}$ and 13:14 prostaglandin reductase which reduces 15-keto $\text{PGF}_{2\alpha}$ to dihydro-15-keto $\text{PGF}_{2\alpha}$. In lung from most species, these enzymes are closely related and the intermediate, 15-keto $\text{PGF}_{2\alpha}$ is not seen in the tissue or in the blood leaving the lung.

Dihydro-15-keto $\text{PGF}_{2\alpha}$, the usual metabolite formed from $\text{PGF}_{2\alpha}$ has been shown to have little spasmogenic activity on many tissues⁷ but the data on 15-keto $\text{PGF}_{2\alpha}$ (ref. 8) are incomplete.

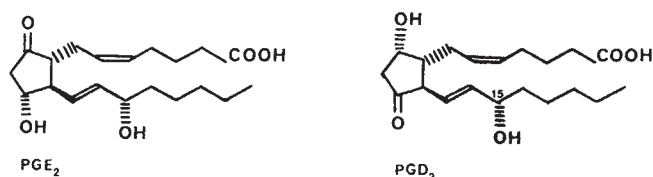


15-keto $\text{PGF}_{2\alpha}$ was synthesised from $\text{PGF}_{2\alpha}$ by oxidation with activated manganese dioxide⁹ and purified by column and plate chromatography; its structure was confirmed by nuclear magnetic resonance and mass spectrometry. Biosynthesis using homogenates of swine lung¹⁰ followed by similar purification gave the same compound.

15-keto $\text{PGF}_{2\alpha}$ was assayed biologically *in vitro* on guinea pig ileum, guinea pig tracheal chains, rat colon, stomach strip and uterus, rabbit aortic strips, gerbil colon and human bronchial muscle strips. Table 1 shows the relative potencies of $\text{PGF}_{2\alpha}$ and 15-keto $\text{PGF}_{2\alpha}$ on these tissues, indicating that 15-keto $\text{PGF}_{2\alpha}$ is more potent than $\text{PGF}_{2\alpha}$ on most preparations. Synthetic and biosynthetic 15-keto $\text{PGF}_{2\alpha}$ were equally active in each test.

PGD_2 , an isomer of PGE_2 and 15-keto $\text{PGF}_{2\alpha}$, was a potent bronchoconstrictor substance, having the same order of potency as $\text{PGF}_{2\alpha}$. This suggests that the 9-hydroxyl group is necessary

¹ Binder, B., Bowler, J., Brown, E. D., Crossley, N. S., Hutton, J., Senior, M., Slater, L., Wilkinson, P., and Wright, N. C. A., *Prostaglandins*, **6**, 87, (1974).



for bronchoconstrictor activity but substitution in the 15 position is less critical. Uncharacteristic responses are, however, occasionally produced by most prostaglandins and although PGE₂ is usually bronchodilator, it sometimes causes bronchoconstriction (H. O. J. Collier and P. Gardiner, symposium on Bronchodilator Drugs, Royal College of Physicians, 1 October, 1973).

All three isomers have been isolated from bovine lung perfusates following immunological challenge. The contraction of both human bronchial muscle strips and guinea pig ileum produced by 15-keto PGF_{2α} is similar in character to that of slow-reacting substance in anaphylaxis (SRS-A), although the substances can be separated by chromatography.

Table 1 Effect of 15-keto PGF_{2α} relative to PGF_{2α} on various isolated smooth muscle preparations

Pharmacological isolated preparation	Relative potency of 15-keto PGF _{2α} (PGF _{2α} = 1)
Guinea pig ileum (11)	2–4
Gerbil colon (11)	5–10
Guinea pig trachea (12)	2–3
Human bronchial muscle (3)	1–2
Rat colon (11)	1–2
Rat stomach strip (11)	0–3*
Rat uterus (11)	0.1–6*
Rabbit aortic strip (11)	†

* Variable responses, with no apparent relationship to concentration.

† No response to either.

Figures in brackets indicate references to methods used.

The belief that prostaglandins are of importance in asthma³ is greatly strengthened by this work, which suggests a definitive involvement of PGs and their metabolites in the asthmatic bronchospasm. Further work to establish the role of 15-keto PGF_{2α} and other metabolites is in progress.

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W. DAWSON
R. L. LEWIS
R. E. MCMAHON
W. J. F. SWEATMAN

*Lilly Research Centre Limited,
Erl Wood Manor,
Windlesham,
Surrey, UK*

Received March 8; revised May 4, 1974.

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Origin of asymmetry in biomolecules

ONE of the most exciting hypotheses put forward to explain the molecular asymmetry of living beings is based on a physical concept, the 'parity nonconservation law'¹. According to this, matter is intrinsically asymmetric (β particles are always spin polarised) and the molecular asymmetry on Earth is a reflection of the structure of matter itself^{2–5}. Though the theory is attractive, experimental results are controversial. Some people have reported differences in the radiation susceptibility of the two enantiomers (D and L forms) of a molecule if irradiated with β^- particles and/or their associated circularly polarised γ rays (bremsstrahlung)⁶, whereas others have failed to detect any differential radiolysis in similar experiments⁷. One possible explanation for the controversial data may be that the decomposition of molecules depends on many factors which may mask the expected differential interaction between β^- particles and optical isomers. To avoid these disturbing factors, we studied the annihilation of β^+ particles in optical isomers, exploiting the great advantage of this technique that it gives information about the interaction process itself rather than about the products of interaction.

The purity of the samples used throughout the experiments was checked by conventional methods. On paper chromatograms with different solvent systems each gave one spot. The absorption, CD and luminescence spectra of the enantiomers were the same between the limit of experimental errors. Sodium-22 was used as the positron emitter. Crystalline D and L amino acids were packed around the ²²Na source (activity 0.8 μ Ci) to a thickness of 6 to 7 mm, and it was placed between two scintillation counters of NE111 plastic phosphor coupled to AVP56 photomultipliers. Conventional electronic circuits selected coincidences between the 1.28-MeV γ radiation of the ²²Na and the 0.51-MeV annihilation radiation produced in the crystals of the two enantiomers. The positrons annihilate on free electrons with a short lifetime⁸ ($\tau \sim 2 \times 10^{-10}$ s) and may form positroniums in singlet ($\tau_s \sim 1.2 \times 10^{-10}$ s) and triplet states ($\tau_t \sim 2.7 \times 10^{-7}$ s) with bound electrons. The triplet positroniums do not, however, live as long in condensed matter, because they transform to the singlet state by the so-called 'pick-off' mechanism with a lifetime of $\tau_{t'}$ ~ 1 to 50×10^{-9} s and annihilate from this state into two photons giving 0.51-MeV γ rays. The time delay was measured by a time-to-amplitude converter and multichannel analyser.

Table 1 Triplet intensity in L and D isomers; data from positron annihilation time spectra

Material	No. of independent runs	L	D	D/L
Phenylalanine (Calbiochem)	8	11.0 (6)	13.4 (6)	0.83 (6)
Tyrosine (Sigma Chemical Corp.)	8	4.4 (2)	4.7 (2)	0.94 (6)
Dihydroxy-phenyl-alanine (Nutritional Biochemical Corp.)	4	1.6 (1)	2.2 (1)	0.72 (6)
Tryptophan ¹ (Nutritional Biochem. Corp.)	4	12.0 (1)	18.0 (1)	0.67 (7)
Tryptophan ² (Koch-Light Laboratories Ltd)	4	18.8 (10)	22.8 (10)	0.82 (5)

The results are summarised in Table 1. There are clearly significant differences in triplet annihilation intensity between L and D amino acids. In other words the experiments show that the D isomers of amino acids favour triplet states in case of forward polarised β^+ particles.

It seems likely from this evidence that β decay was the cause of an initial asymmetry in the racemic mixtures present on the primordial Earth. This tiny initial asymmetry was probably enough, in the course of chemical and biological evolution, to nudge the system into a highly asymmetric state by one or other of the mechanisms suggested by Wald⁹ and others¹⁰⁻¹².

For the physical interpretation of the effect we rely on the fact¹³ that the symmetry properties of optical isomers are compatible with the existence of second rank pseudotensors characterising the isomers. The correlation tensor $\langle v_\alpha \sigma_\beta \rangle$ between the velocity and the spin of the electrons of the sample is of this type. Therefore those components of the correlation tensor which are allowed by the symmetry may differ from zero. In a solution, for example, the correlation tensor has the form,

$$\langle v_\alpha \sigma_\beta \rangle = K \sigma_{\alpha\beta}$$

where the coefficient K is of different sign in L and D species. In other words, when $K \neq 0$ the electron spins are aligned predominantly parallel to the motion in one of the optical isomers, and antiparallel in the other.

If we suppose that the probability of positronium formation depends on the relative velocity of the positrons and the electrons, then since the positrons are polarised this assumption immediately leads to the consequence that when $K \neq 0$, the relative probabilities of *ortho*- and *para*-positronium formation differ for differential optical isomers. This difference is indeed observed in the experiment described above.

A. S. GARAY

Biological Research Center,
6701 Szeged, POB 521, Hungary

L. KESZTHELYI
I. DEMETER
P. HRASKO

Central Research Institute for Physics,
1121 Budapest, Hungary

Received July 23, 1973; revised February 11, 1974.

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Abnormality in tissue isoferitin distribution in idiopathic haemochromatosis

IDIOPATHIC haemochromatosis (IHC) is generally considered to result from an inherited abnormality of iron metabolism in which excess iron accumulates in tissues such as liver, heart and pancreas, causing structural and functional abnormalities¹. The clinical and pathological features of the disease have been well characterised^{1,2} although the mode of inheritance is uncertain because of the lack of a reliable genetic marker. In spite of several theories³⁻¹¹, the

metabolic abnormality leading to excess iron storage remains unknown.

Much of the excess iron is deposited in ferritin. Although the major function of this protein was thought to be the detoxification and storage of iron, it now seems to have a more extensive function. For example, it acts catalytically in the oxidation and storage of iron^{12,13}, and there is evidence¹⁴⁻¹⁶ for metabolically different ferritins. We found a structural heterogeneity in many tissue ferritins which may correlate with this functional heterogeneity. Most tissues from normal subjects contain several isoferitins, many of which are common to several tissues. The relative distribution and iron content of these isoferitins are characteristic of the tissue of origin (our unpublished results). Because of this evidence of multiple organ-specific forms and metabolically distinct pools of ferritin, and also the demonstration of an alteration in isoferitin profile in human hepatoma¹⁷, we have examined ferritin isolated from liver and other organs in patients with IHC and in control subjects. We found an apparent absence of the more acidic isoferitins that predominate in normal heart, pancreas and kidney ferritin. The haemochromatotic tissue ferritins all closely resembled normal liver ferritin in their isoferitin profile, iron content of the isoferitins and in amino acid composition. By contrast, tissue ferritins isolated from patients with alcoholic cirrhosis or secondary iron overload showed the normal organ distribution in isoferitin profile and in iron content.

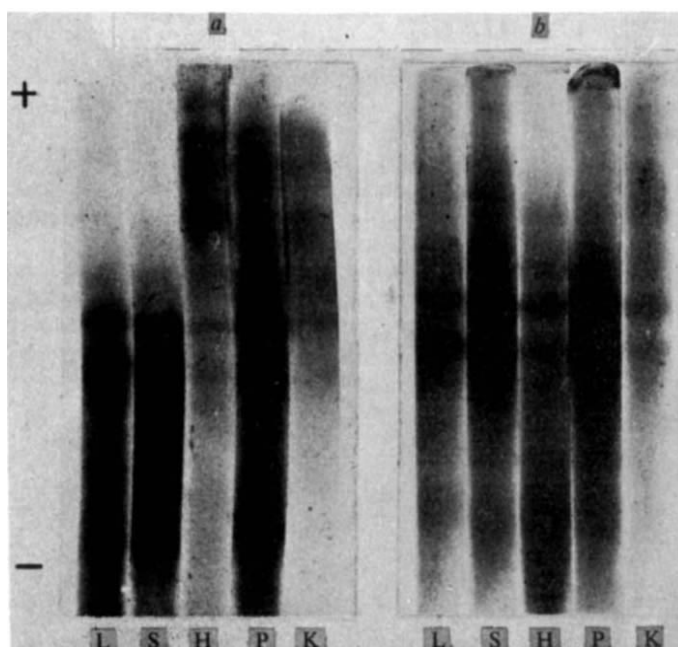


Fig. 1 Gel electrophoresis in 4% polyacrylamide using 2% ampholytes, pH range 5-7, of purified ferritin isolated from human liver (L), spleen (S), heart (H), pancreas (P) and kidney (K) from a normal subject (a) and a patient with idiopathic haemochromatosis (b). Between 25 and 35 μ g of protein was applied to all gels. After equilibrium was attained, the gels were stained for protein using Coomassie blue.

Fresh human tissues were obtained post-mortem from three patients with IHC (all untreated and diagnosed according to published criteria¹⁸), two with alcoholic cirrhosis, one with post-transfusional haemosiderosis and two control subjects. Ferritin was isolated by the method of Drysdale and Munro¹⁹ except that carboxymethyl cellulose chromatography was omitted to avoid possible selective loss of isoferitins of differing charge. In contrast to methods which include high speed centrifugation for purification, this procedure ensures the collection of both iron-laden ferritin and the lighter apoferritin, which is important in

Table 1 Amino acid composition of normal and haemochromatosis tissue ferritin

Amino acid	Normal			Haemochromatosis		
	Liver	Spleen	Kidney	Liver	Spleen	Kidney
Lys	13.1	13.8	9.15	12.3	13.3	12.5
Hist	6.6	7.4	4.9	7.1	7.1	6.3
Arg	8.6	9.8	7.2	10.3	9.6	8.2
Asp	20.1	19.5	19.7	21.1	21.4	21.0
Thre	8.5	9.3	9.15	7.3	7.7	7.7
Ser	10.0	10.3	10.45	8.2	6.9	9.6
Glu	22.9	18.3	22.5	22.8	20.6	23.9
Pro	7.1	7.6	10.3	5.3	6.3	5.1
Gly	13.1	13.0	16.2	12.5	13.9	12.9
Ala	15.2	15.4	15.1	16.1	16.2	15.8
Cyst*	2.1	1.7	4.9	2.6	2.3	2.0
Val	8.9	9.8	10.4	7.4	8.3	7.3
Meth†	3.2	1.8	3.5	3.5	2.2	3.9
Ileu	4.0	3.5	5.7	4.0	3.5	3.6
Leu	20.9	23.1	18.8	24.6	23.7	24.3
Tyr	5.6	7.2	5.2	6.2	7.0	6.7
Phe	7.6	8.3	7.0	8.1	7.9	8.7

Tissue ferritins were isolated and purified as described in the text. The results are expressed as residues 180 amino acid subunit and are means of at least two duplicate determinations on each sample.

* Determined as cysteic acid plus half cysteine.

† Includes methionine and methionine sulphoxide.

view of the variable iron content of unfractionated ferritin¹⁹ and its multiple molecular forms (our unpublished results). Each tissue ferritin preparation appeared to be pure by gel electrophoresis since all protein in each preparation also stained for iron with Prussian blue, a commonly accepted criterion for ferritin purity.

The tissue ferritin preparations were also analysed by gel electrofocusing (GEF) at 4° C in 4% polyacrylamide gel cylinders with 2% ampholytes, pH range 5–7, in apparatus from Medical Research Apparatus, Boston^{20,21}. The gels were stained for protein with Coomassie blue or for iron using potassium ferrocyanide. To establish that all protein bands obtained from each ferritin preparation by GEF represented isoferritins and not other contaminating proteins, duplicate gels were subjected to direct immunoprecipitation *in situ* with a specific rabbit anti-human-liver ferritin antiserum. This antiserum recognised all the tissue ferritins with a line of complete identity by Ouchterlony

analysis and, in addition, precipitated all the isoferritins previously demonstrated in the various normal tissues (our unpublished results). The resulting pattern of immunoprecipitates after GEF corresponded in position in each instance to the protein bands (our unpublished results), confirming that all protein bands were ferritins.

In agreement with our previous findings (unpublished), there were characteristic differences in electrophoretic mobilities of the ferritins from normal tissues. These differences were more evident at the higher resolution given by GEF. There were striking differences between the tissue ferritins in the isoferritin distribution and the iron content of the individual isoferritins (Figs 1 and 2). Thus, the number and relative amounts of various isoferritins, as well as their iron content varied with the tissue of origin. This organ-specific variation in isoferritin pattern and iron content was also demonstrable in ferritins isolated from the tissues of the two patients with cirrhosis and one with transfusional haemosiderosis. In contrast, ferritins isolated from each of the five tissues from three patients with IHC were remarkably uniform in electrophoretic mobility, isoferritin profile and the iron content of their isoferritins (Figs 1 and 2). These results were substantiated by amino acid analyses which showed that the composition of different tissue ferritins in IHC were very similar and closely resembled that of normal liver ferritin. This contrasts with the variation in amino acid compositions of normal tissue ferritins (our unpublished results). Immunodiffusion experiments using five animal antisera to heterologous ferritins revealed no immunological differences between normal liver ferritin and the ferritins isolated from haemochromatotic tissues when tested by double diffusion on Ouchterlony plates.

The electrophoretic mobilities and isoferritin profiles of tissue ferritins from a patient with post-transfusional haemosiderosis were normal. Alfrey obtained similar results by electrophoresis with two patients with post-transfusional haemosiderosis²³. Linder-Horowitz *et al.*¹⁶ demonstrated that experimental iron loading in rats did not alter the gene expression for ferritin. It is unlikely, therefore, that the abnormalities in tissue ferritins which we have demonstrated in patients with IHC are merely the result of increased iron concentration. Rather, the atypical isoferritin distribution seems to be specific for this disease.

There is increasing evidence that the structural heterogeneity in different ferritins from normal tissues reflects functional differences in these proteins in iron metabolism^{14,16,23}. Variations in isoferritin profile among normal tissue ferritins may be due either to the presence of different cell populations in the different tissues, or to different metabolic functions of the multiple isoferritins. Recent work²⁴ has suggested that much of the structural heterogeneity in tissue ferritins represents hybrid molecules consisting of different proportions of dissimilar subunits, somewhat analogous to other hybrid isoenzymes such as lactic dehydrogenase. The abnormally uniform distribution of tissue isoferritins which we have demonstrated in IHC could result from the generalised deposition of a catabolic or storage ferritin^{14,15} normally present predominantly in liver and spleen or from a decreased synthesis of the more acidic isoferritins present in heart, kidney and pancreas. This may represent a genetic defect in the synthesis of a subunit type found normally in these tissue ferritins. Whether the abnormality in tissue isoferritins in IHC is directly related to the primary genetic defect of this disease requires confirmation by further studies of asymptomatic relatives of patients and more detailed analysis of the primary structure of ferritin.

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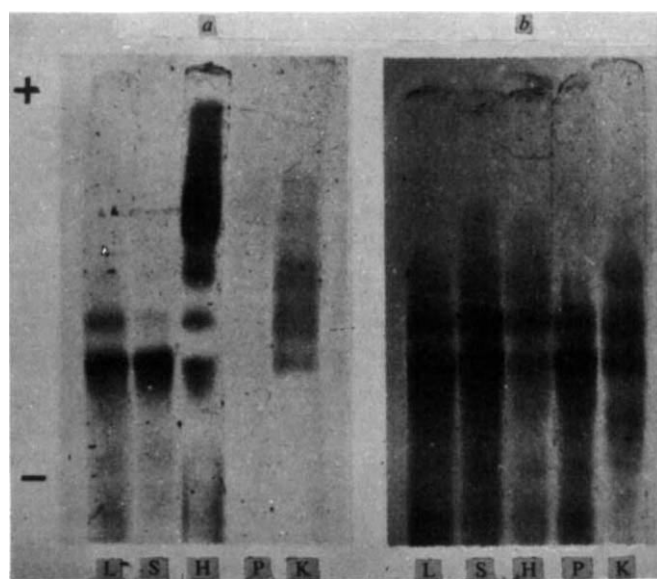


Fig. 2 Analytical gel electrofocusing was performed as described in Fig. 1. In this case, the gels were stained for iron using Prussian blue. The isoferritins in normal pancreas did not stain for iron, even when the gels were relatively overloaded with protein. *a*, Normal; *b*, haemochromatosis.

LAWRIE W. POWELL
ELLIOT ALPERT
KURT J. ISSELBACHER

Departments of Medicine,
Harvard Medical School, and
Departments of Medicine,
(Gastrointestinal Unit),
Massachusetts General Hospital,
Boston, Massachusetts 02114

JAMES W. DRYSDALE

Department of Biochemistry
and Pharmacology,
Tufts University School of Medicine,
Boston, Massachusetts 02114

Received February 11; revised June 10, 1974.

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A phenylethylamine oxidising defect in migraine

FOR some two hundred years, certain foodstuffs have been known to trigger migraine attacks¹. In 1967, knowing that cheese is a common dietary trigger to migraine, Hanington² fed tyramine, which it is known to contain, to affected subjects and by this action was able to initiate headache episodes.

Even though cheese and tyramine-containing foods are frequently implicated as headache precipitants, by far the commonest of the dietary triggers is chocolate³ which does not contain tyramine according to a recent analysis performed by the British Food Manufacturing Industries Research Association. This analysis did however reveal the presence of large amounts of phenylethylamine, at least 3 mg per 2 ounce bar; of the other common dietary precipi-

Table 1 Effect of phenylethylamine in chocolate-sensitive migraine

	Lactose	Phenylethylamine
Headache	6	18
No headache	30	18

tants tested, a large number of cheeses, but not all, contained phenylethylamine, as do some red wines (M. J. Saxby, personal communication). It therefore seemed of interest to determine whether oral phenylethylamine alone is able to initiate an attack of migraine in susceptible subjects.

Forty-six migraine sufferers who claimed, in answer to a questionnaire, that their headache attacks were provoked by chocolate, volunteered to take part in the following sighting experiment, which was organised along similar lines to that reported earlier for tyramine².

A capsule containing lactose was sent to each, together with a further questionnaire to be completed and returned 24 h after it had been ingested. Those taking part were asked not to take the capsule within 48 h of a spontaneous migraine attack or when they thought one might be expected. After this questionnaire had been returned, a capsule similar to the earlier one but containing phenylethylamine (3 mg) was sent, together with another questionnaire and similar instructions for its return. Patients were not aware of the contents of either capsule.

Ten subjects failed to complete the trial after lactose ingestion only, which had been followed by headache in five of them. Thirty-six completed the trial, 3 men and 33 women. The results are shown in Table 1. Included in this table are two patients who developed a headache after both lactose and phenylethylamine. One had a mild generalised headache on both occasions but felt sick only after phenylethylamine; the other had a unilateral mild to moderate headache after both. Of the four subjects who had symptoms only after lactose one had no headache at all but saw patterns before her eyes, two had a moderately severe unilateral headache and the fourth a bilateral moderately severe headache.

There seems to be a *prima facie* case for the existence of a cause and effect relationship between phenylethylamine ingestion and migraine headache in certain patients.

One unexplained feature of the phenylethylamine-provoked headaches is that their onset was delayed for approximately 12 h after amine ingestion, a period of the same order as that observed clinically after chocolate

Table 2 Human platelet monoamine oxidase activity

Substrate	Migraine	Patient Control	Chocolate-sensitive migraine
Phenylethylamine	4.38±0.51 (28)	10.66±0.96 (26)	4.16±0.36 (12)
	$P < 0.001$		$P < 0.001$
Tyramine	13.52±1.60 (28)	28.48±2.64 (26)	11.80±1.26 (12)
	$P < 0.001$		$P < 0.001$
Dopamine	5.61±0.51 (28)	8.29±1.09 (26)	5.04±0.44 (12)
	$P < 0.05$		$P < 0.01$
5-Hydroxytryptamine	14.54±1.17 (16)	21.70±1.82 (14)	15.84±1.88 (7)
	$P < 0.01$		$P < 0.05$

Each value is expressed as nmol of substrate deaminated per 30 min incubation per mg protein at 37° C. Protein nitrogen was measured according to the method of Lowry *et al.*²⁴ using human serum albumin as standard. Figures in parentheses represent number of subjects.

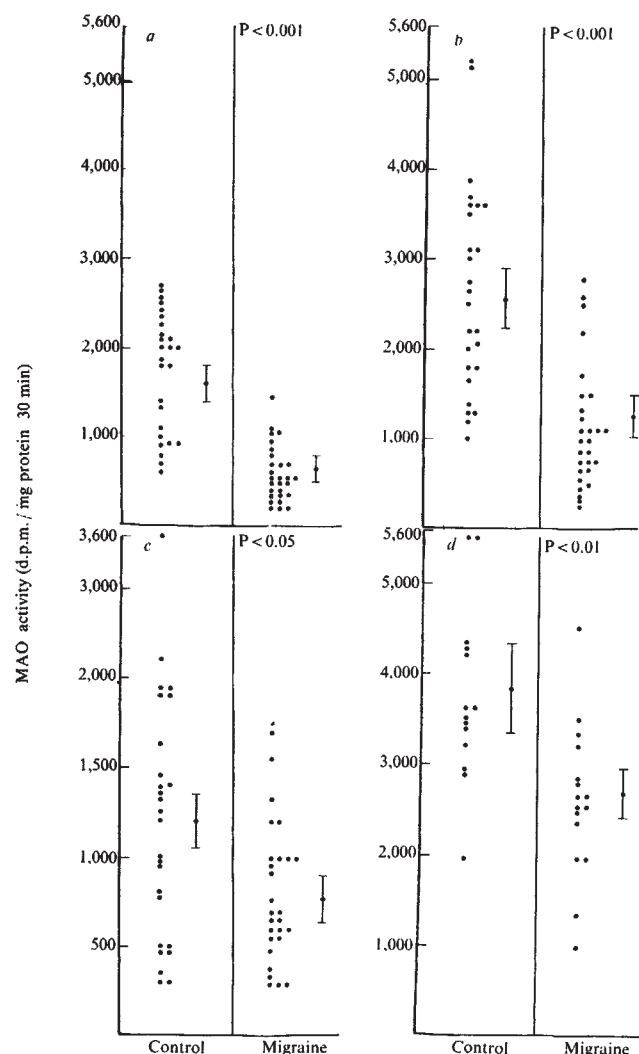


Fig. 1 Distribution of monoamine oxidase activity in blood platelets from migrainous and control subjects. Activity is expressed as d.p.m. radioactive deaminated metabolites formed per mg protein per 30 min using *a*, phenylethylamine; *b*, tyramine; *c*, dopamine; and *d*, 5-hydroxytryptamine as substrates.

ingestion in susceptible subjects. During the earlier experiment with tyramine² two groups of reactors were observed, with peak onset at 3 h and 12 h respectively. The dose of tyramine used then (100 mg) was considerably larger than that of phenylethylamine (3 mg) in the present trial. A 12 h lag period is puzzling and at first sight, seems to rule out simple trigger action such as that envisaged by one of us⁴: taking as his starting point some published observations⁵ in which tryptamine and 5-hydroxytryptamine, when introduced into the afferent pulmonary circulation of an experimental animal, had been shown to cause a release of prostaglandins and other vasoactive agents into the systemic circulation, he suggested that migraine might, in a sense, be a pulmonary disease. A triggering agent arriving at the lungs from the gastrointestinal tract might, as in the perfused animal lung, provoke a secondary release of vasoactive substances to act on the cerebral vasculature. This interpretation has received a measure of experimental support⁶. Recently Y. S. Bakhle (personal communication) has found phenylethylamine to be an effective releaser of vasoactive substances in certain species, rather more so than tyramine⁷.

If Sandler's hypothesis⁴ were valid, one possible explanation to account for a facilitated release of vasoactive substances from the lungs into the systemic circulation would be the access of a higher concentration of monoamine into the afferent pulmonary vessels brought about by defective

inactivation. The predominant inactivation pathway of phenylethylamine involves oxidative deamination by monoamine oxidase (MAO)⁸. There is evidence of decreased platelet MAO activity during headache episodes in migrainous patients^{9,10}.

Johnson¹¹, working with enzymes from rat brain, obtained data which he interpreted in terms of the existence of two types of MAO, termed A and B, and similar conclusions have been drawn from findings on human neural tissue¹². It has been claimed that these forms are immunologically separable¹³. Type A is specific for 5-hydroxytryptamine¹¹ and noradrenaline¹⁴; tyramine¹¹ and dopamine¹⁵ are substrates for both A and B forms. Phenylethylamine is a preferred substrate for MAO B (ref. 16). It therefore seemed to us that, in migraine, we might be dealing with a specific defect of MAO B. To test this hypothesis, a study of platelet MAO activity, using several different substrates, was carried out in a further series of migrainous patients and in non-migrainous controls.

During a headache-free period, venous blood samples from the unselected group of 28 migrainous subjects (9 male, 19 female), age range 20 to 65 yr, were collected into plastic tubes containing lithium heparin. Nonmigrainous volunteers of a similar age range provided control samples. Platelets were collected¹⁷ and stored deep-frozen before assay. MAO activity was measured radiometrically¹⁸ using ¹⁴C-labelled phenylethylamine, tyramine, dopamine and 5-hydroxytryptamine as substrates.

The results are shown in Fig. 1 and Table 2. A highly significant decrease in phenylethylamine oxidising ability was observed in migraine compared with control subjects, but in addition, a difference in tyramine oxidation of a similar order was apparent between the two groups. A smaller but still significant decrease in activity towards dopamine was present. The deficit of MAO A may be less pronounced, for there was a small but significant decrease in 5-hydroxytryptamine-oxidising ability in the migrainous group. In retrospect the decrease in the normally high¹⁷ benzylamine-oxidising ability in platelets from migrainous subjects, observed by another group¹⁰, is compatible with our data on impaired phenylethylamine oxidation, for benzylamine seems to be another substrate of MAO B (ref. 11).

It seems likely, however, that the A and B classification is a gross over-simplification^{19,20}: one of the multiple forms of solubilised MAO which has been isolated electrophoretically²¹ seems to possess, for instance, an extremely high preference for dopamine as substrate. Nevertheless, whichever of the multiple forms of MAO turns out to be implicated in migraine, it seems quite possible that the deficit of phenylethylamine and tyramine-oxidising ability is of functional significance.

The migrainous patients under investigation were a mixed group, some with headache provoked by chocolate or other foodstuff and others without history of dietary precipitant. Platelet samples obtained from patients with dietary compared with non-dietary migraine variants, however, showed no significant difference in their ability to oxidise any of the substrates investigated (Table 2). Before firm conclusions can be drawn from data of this type, more information is needed showing to what extent any platelet MAO defect reflects a similar deficit in small intestinal or perhaps, pulmonary MAO A pulmonary deficit, for instance, would make larger concentrations of amine available locally, thus helping the threshold to be reached for an 'all-or-none' release²² of vasoactive substances from the lung into the systemic circulation in accordance with Sandler's hypothesis⁴. Were this hypothesis to prove invalid, an alternative and simpler explanation might be that monoamines which escape destruction in the lungs²³ because of an MAO deficit exert some direct action on the cerebral vasculature. Preliminary observation (R. J. Stephens, personal communication), however, indicate that phenylethylamine has little or no direct effect

of this type, at least in the rat.

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M. SANDLER

Bernhard Baron Memorial Research Laboratories
and Institute of Obstetrics and Gynaecology,
Queen Charlotte's Maternity Hospital,
London W6 0XG, UK

M. B. H. YODIM

Medical Research Council Clinical Pharmacology Unit,
Radcliffe Infirmary,
Oxford OX2 6HE, UK

EDDA HANINGTON

The Wellcome Trust,
52 Queen Anne Street, London W1M 9LA, UK

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to inhibition of cell multiplication by serum limitation^{4,5}. In addition, different fractions of serum stimulate the growth of untransformed mouse 3T3 fibroblasts and 3T3 cells transformed by SV40 (SV3T3) in culture⁶ which suggests the involvement of different growth promoting molecules for normal and transformed cells. It has been shown that a new pituitary hormone, fibroblast growth factor⁷ (FGF), with a glucocorticoid, hydrocortisone, and with a nonspecific carrier protein could completely replace exogenously added serum for the induction of the complete mitogenic response in lines of BALB/c 3T3 cells⁷, whereas virtually no effects were observed on virally transformed cells (P. S. R., W. S. and D. G.; manuscript in preparation). In the presence of serum exhausted for its growth-initiating ability only FGF was required to initiate or increase DNA

Table 1 Additions to growing cultures

Additions	3T3		ts 3T3 Cl.1		Py3T3	
	a, c.p.m.	b, cells	a, c.p.m.	b, cells	a, c.p.m.	b, cells
32°C						
None	1.4	4.4	2.4	4.2	2.2	6.5
FGF	6.1	10.8	2.2	4.3	2.3	6.7
Serum	7.1	12.8	3.9	6.9	4.1	10.4
39°C						
None	1.6	4.0	2.0	4.1	6.5	2.1
FGF	7.6	6.6	9.4	6.6	5.6	2.0
Serum	6.0	7.1	9.7	6.7	9.3	2.9

BALB/c 3T3 cells¹⁵, polyoma transformed BALB/c cells (Py3T3), and temperature-sensitive transformed BALB/c 3T3 cells (ts 3T3 Cl.1) were plated at 10^5 per 5 cm Petri dish and grown in Dulbecco's Modified Eagles Medium (5 ml) in 2% calf serum (Colorado) at 32° or 39° in a 10% CO₂ atmosphere. The CO₂ pressure was slightly varied to maintain a constant pH in the medium at the two temperatures. After 2 or 3 d when the final cell density was about 3×10^5 per plate (3T3: 3.0 and 3.1; ts 3T3: 2.9, 3.0; and Py3T3: 1.6 and 4.5×10^5 cells at 39°C or 32°C respectively) 50 ng ml⁻¹ of FGF or 10% serum (6 mg ml⁻¹) were added to the growing cultures. Autoradiography of control dishes without additions showed that more than 70% of all cell nuclei became radioactively labelled during the first 12 h after the time of addition of ³H-thymidine. Cultures were radioactively labelled with 3 µCi ml⁻¹ ³H-methyl thymidine at 3 µM from 7 until 30 h after additions, or the cells were counted in a Coulter Counter 48 h afterwards. The c.p.m. of ³H-thymidine incorporated into DNA (ref. 16) per 10 cells (a) or the number of cells per 5 cm Petri dish $\times 10^{-3}$ were recorded (b). FGF, a homogeneous polypeptide of molecular weight 13,400 was purified from bovine pituitary glands as described previously⁷.

synthesis and cell division; the hydrocortisone concentration present in serum was considered to be sufficiently high not to require further addition⁷. We have now investigated the ability of FGF to promote the growth of cell mutants of BALB/c 3T3 which show temperature dependent changes in many properties characteristic of the transformed state (W. E., manuscript in preparation). We found that FGF initiates DNA synthesis and cell multiplication only at the non-permissive temperatures ('normal' state) but fails to initiate or promote cell growth at permissive temperatures (transformed state).

Cell mutants with temperature-sensitive (ts) lesions in many properties characteristic of transformed cells have been described for SV3T3⁸ and baby hamster kidney⁹ (BHK) cells, similar to the earlier reports of temperature sensitive variants of BHK obtained by transformation with a thermosensitive mutant of polyoma virus¹⁰. Recently Eckhart has selected several different clones of BALB/c 3T3 cells which show temperature dependent properties. Properties of the three mutant clones used in this paper are stable and can be summarised. Cells from these clones show transformed morphology and ability to grow in agar at 32° C but normal morphology and lack of growth in agar at 39° C (W. E., unpublished observations). Growth rates are 1.8, 2.0 and 1.7 times as fast at 32° C as at 39° C in medium containing 10% serum. Their final cell densities in medium containing low serum concentrations are approximately 10 to 3-fold higher at 32° C than at 39° C (for example for ts 3T3 clone 1 in 0.25%, 0.5% or 2% serum

Cell transformation mutants are not susceptible to growth activation by fibroblast growth factor at permissive temperatures

CHEMICAL¹ or viral² transformation of fibroblasts in tissue culture results in cell variants with different properties from the original cells (see ref. 2 for review). These properties include morphological changes, surface alterations³ and an increased growth ability under various conditions. This changed pattern in growth control of many transformed cells is manifested *in vitro* by their ability to grow in agar, to attain higher saturation densities in monolayer cultures and to be much less responsive

Table 2 Stimulation of DNA synthesis in different cells

Additions	3T3	ts Cl.1	ts Cl.10	ts Cl.X	Py3T3
32° C					
None	1.1	0.5	13.8	8.0	46.0
FGF	110	0.8	15.8	10.2	42.3
39° C					
None	1.0	1.2	0.8	1.2	70.4
FGF	85.6	64.5	42.5	56.8	59.2

Abbreviations: 3T3; BALB/c 3T3; ts Cl.1, Cl.10, Cl.X: temperature sensitive transformed BALB/c 3T3 cell-lines; Py3T3: polyoma transformed BALB/c 3T3. The percentage of ^3H -thymidine incorporated into DNA compared with serum addition is shown. Cells were grown in DEM and 10% serum as described (Table 1) at either 32° C or 39° C until confluent (10^6 cells per 5 cm plate). Medium was then removed, cell monolayers were washed twice with DEM containing $250 \mu\text{g ml}^{-1}$ bovine serum albumin (BSA) and fresh DEM containing 0.2 ng ml^{-1} FGF, $0.5 \mu\text{g ml}^{-1}$ hydrocortisone (cortisol, Calbiochem) and $250 \mu\text{g ml}^{-1}$ BSA. After 3 d 50 ng ml^{-1} of FGF or 10% serum (6 mg ml^{-1}) was added and the cultures were radioactively labelled with ^3H -thymidine from 8 until 34 h after additions, the c.p.m. of ^3H -thymidine incorporated into DNA was recorded. Results for no addition or the addition of FGF are expressed as the percentage of the c.p.m. incorporated into DNA in the presence of 10% serum. Autoradiography of parallel cultures showed that the percentage of radioactivity labelled nuclei in the population with FGF addition were: for 3T3, 95 and 90; for ts 3T3 Cl.1, 1 and 60; for Cl.10, 10 and 40; for Cl.X, 7 and 51; for Py3T3, 40 and 53 at 32° C and 39° C respectively. Without addition of 50 ng ml^{-1} FGF the percentage of radioactively labelled nuclei was less than 1% for 3T3, ts 3T3 Cl.1 at 32° C and 39° C, Cl.10 and Cl.X at 39° C while for the remainder: Cl.10, 7%; Cl.X, 4% at 32° C; Py3T3, 41% at 32° C and 60% at 39° C. The percentages of the cell population which had divided by 48 h after addition of 50 ng ml^{-1} of FGF were for 3T3, 96 and 86; for Cl.1, 4 and 82; for Cl.10, 16 and 87; for Cl.X, 9 and 88; for Py3T3, 50 and 54 at 32° C and 39° C respectively.

the final cell densities were 19 and 2.2, 20 and 3, 32 and 11×10^5 cells per 5 cm dish at 32° C and 39° C respectively). At low serum concentrations (Table 2), ts 3T3 Cl.1 and to a lesser extent Cl.10 and Cl.X can be made to exhibit density-dependent inhibition of growth at 32° C similar to nontransformed 3T3 cells and unlike the virally transformed lines Py3T3 and SV3T3³. After addition of 20% serum to such quiescent cultures at 32° C they synchronously initiate DNA synthesis followed by cell division at times compatible with an original cell population arrested in the G_0 phase of the cell cycle (unpublished results).

Addition of FGF to cultures of BALB/c 3T3 growing slowly with 2% serum in the medium increased both rates of DNA synthesis and cell division but failed to increase growth rates of polyoma transformed 3T3 cells (Py3T3) (Table 1). For ts-3T3 clone 1 (ts-Cl.1) cells addition of FGF specifically increased cellular growth rates at 39° C but not at 32° C. 10% serum addition markedly increased DNA synthesis and cell division in all cultures (Table 1). The ability of FGF to initiate DNA synthesis in quiescent cultures was also checked. It was however impossible to maintain resting cultures of BALB/c 3T3 cells with Dulbecco's modified Eagle's medium (DEM) and only BSA, and hence low concentrations of FGF (0.2 ng ml^{-1}) and hydrocortisone were added to prevent cell deterioration¹¹. Addition of saturating amounts of FGF (50 ng ml^{-1}) to quiescent cultures of 3T3, ts Cl.1, Cl.10, Cl.X at 39° C induced a 40- to 80-fold increase in DNA synthesis but virtually no increase in resting cultures of ts Cl.1 at 32° C. It was more difficult to obtain completely quiescent cultures of ts Cl.10 and Cl.X at 32° C, but FGF failed to increase the small residual amount of DNA synthesis (Table 2). Py3T3 cells continued synthesising DNA at half the rates of those in 10% serum but no additional stimulation was observed upon FGF addition (Table 2). Approximately twice the amount of FGF was added to the cultures in Tables 1 and 2 than was required for complete induction of DNA synthesis and cell division in resting cultures of BALB/c 3T3 cells (manuscript in preparation). Further increased additions of FGF up to five times the amounts added in Table 2 failed to stimulate DNA synthesis in mutant cultures maintained at 32° C,

whereas 10% serum addition induced more than 95% of the cells to synthesise DNA and divide.

The ability of various hormones to promote the initiation of DNA synthesis in resting cultures of ts Cl.1 at either 32° C or 39° C is shown in Table 3. Insulin at high, nonphysiological concentrations was reported to induce DNA synthesis and cell division in fibroblasts maintained in the presence of serum^{13,14}. But in the absence of serum, insulin at physiological (Table 3) (10^{-9} to 10^{-8} M) or nonphysiological (10^{-6} M) (unpublished results) concentrations failed to induce DNA synthesis in more than 5% of the cell populations at either temperature. FGF without hydrocortisone still induced DNA synthesis in a significant fraction (22%) of the mutant cells at 39° C but not at 32° C. With hydrocortisone the induction of DNA synthesis was comparable to that with serum at 39° C while only serum induces DNA synthesis at 32° C.

A comparative biochemical study of normal and transformed cells in tissue culture is complicated by the fact that often two very different cell types are being compared (for example,

Table 3 Induction of DNA synthesis by different hormones

Additions	^3H -DNA (c.p.m. $\times 10^{-5}$)		Labelled nuclei (%)	
	32° C	39° C	32° C	39° C
none	0.5	0.2	1.8	1.0
HC	0.7	0.2	2.6	1.2
FGF	0.7	5.8	2.8	22.7
insulin	0.4	0.8	2.1	3.0
HC + FGF	0.4	10.8	3.1	66.2
HC + insulin	0.3	0.3	2.0	1.6
HC + FGF + insulin	0.7	9.9	3.2	67.2
serum	12.0	11.5	92.5	71.7

Cell cultures of the temperature sensitive transformed BALB/c 3T3, clone 1 were plated and grown as described in Table 2. At confluency (10^6 cells per 5 cm dish) the medium was removed and the cell monolayers washed twice as described. Medium containing 0.2 ng ml^{-1} FGF and $250 \mu\text{g ml}^{-1}$ BSA was added back and the cultures incubated at their respective temperatures for another 3 d. To the resting cultures either $0.5 \mu\text{g ml}^{-1}$ of hydrocortisone (HC), 50 ng ml^{-1} fibroblast growth factor (FGF), 50 ng ml^{-1} insulin (bovine pancreas, Calbiochem) or 10% serum was added as indicated. Cultures were radioactively labelled with either $3 \mu\text{Ci ml}^{-1}$ ^3H -thymidine at $3 \mu\text{M}$ for DNA synthesis or at $1 \mu\text{M}$ for autoradiography, from 10 until 34 h after additions. The c.p.m. of ^3H -thymidine incorporated into DNA $\pm 10^{-5}$ per culture or the fraction of cells with radioactively labelled nuclei is shown for various hormone additions. Cell numbers were 9.2 and 9.3×10^{-5} cells per 5 cm dish at 32° C and 39° C respectively before the additions, and FGF + HC addition increased cell numbers to 9.4 and 17.0×10^5 at 32° C and 39° C respectively measured after 48 h.

chromosomal differences in SV3T3 and 3T3), and the observed differences could arise as a result of the transformation event itself or as a consequence of the selection for a particular stable transformant. With cells possessing thermosensitive lesions in the expression of the transformed phenotype, however, the latter difficulty is obviated. We now show that the growth-initiating properties of a mutant 3T3 cell can be controlled by a single purified polypeptide hormone at the nonpermissive temperature; this ability is lost at the permissive temperature when the cells exhibit many of the properties characteristic of the transformed state. Other components in serum can control the growth at this permissive temperature. The cellular site of action of many of the polypeptide hormones is the adenyl cyclase in the plasma membrane¹⁴. We have previously shown that FGF can directly activate the membrane-bound guanyl cyclase in 3T3 but not in SV3T3 cells (manuscript in preparation). This suggests that the temperature-sensitive lesion in the mutant cells is either wholly or in part caused by a membrane change. This change possibly prevents the polypeptide hormone from elevating intracellular cGMP concentrations to a level which may be responsible for the initiation of cell growth¹¹.

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P. S. RUDLAND*
W. ECKHART
D. GOSPODAROWICZ
W. SEIFERT

The Salk Institute for Biological Studies,
P.O. Box 1809,
San Diego, California 92112

Received March 29, 1974.

*Present address: Division of Cell Regulation, Imperial Cancer Research Fund, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX.

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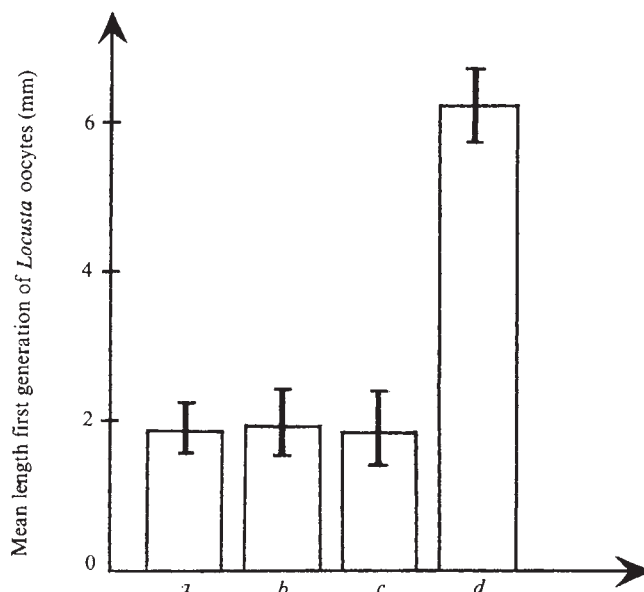


Fig. 1 Mean length (mm) of the first generation of oocytes in *Locusta*, on day 10 of imaginal life, after various electrical stimulations. a, Controls (electrode placed in the PI without passage of current; 16 animals, 1 died); b, stimulation of the ventral nerve cord; (16 animals, 2 died); c, stimulation of the deutocerebrum (16 animals, none died); d, stimulation of the PI (17 animals, 2 died, 2 laid one batch of eggs).

introduced into the PI and a thoracic indifferent electrode. After stimulation, the dorsal fin tegumentary window is closed. It is thus possible to achieve a stimulation every 48 h; each individual was subject to two stimulations (*Locusta*) or three (*Anacridium*). The results were compared with those obtained in the controls subjected to the introduction of an electrode without passage of current, the stimulation of the first abdominal ganglion, and stimulations of various regions of the brain (deutocerebrum in *Locusta*, tritocerebrum in *Anacridium*). The conditions were the same as for the operated animals. A parallel histological study enables verification of the placement of the electrode and the emptying of the cell bodies of the PI after stimulation. The results were expressed in terms of the length of the first oocyte generation 8 d (*Locusta*) (Fig. 1) or 28 d after the first stimulation (*Anacridium*) (Fig. 2).

In *Locusta*, stimulation of the PI is accompanied by a rapid growth of the oocytes. The first generation oocytes on day 10 have an average length of more than 6 mm (Fig. 1d); in all the controls (Fig. 1a, b and c), this length remains below 2 mm. This rapid growth even induced 2 of the 15 stimulated animals to lay eggs before day 10; in the usual breeding conditions egg laying occurs only about day 18.

In the case of *Anacridium*, the adult females used were about 2 month old and in natural conditions would have laid eggs only 6 months later. The triple stimulation of the PI provoked a partial emptying of the cell bodies of the PI and rupture of the ovarian diapause. All the animals laid eggs at least once in the 28 d following the first stimulation; one of them even laid twice. The first oocyte generation at this time had an average length close to 7 mm, so the eggs were mature (Fig. 2d). In all the controls (Fig. 2a, b and c) the average length of this same generation is less than 2 mm and storage of the protocerebral neurosecretion was observed^{4,5}.

Finally, it is possible to associate the stimulation method with that of ablations. Thus, in the case of *Anacridium*, stimulation of the PI of allatectomised animals results in almost zero growth of the oocytes (Fig. 2e): the gonadotrophic role of the CA is thus confirmed. Conversely, ani-

Manipulation of sexual physiology by brain stimulation in insects

IN insects the pars intercerebralis (PI) is a median protocerebral neurosecretory centre associated with postcerebral neurohaemal organs, the corpora cardiaca (CC). Numerous investigations based on ablations or implantations of the PI and CC have shown that the neurohormones released by the CC intervene in many physiological processes. *In vitro*, by means of electrical stimulations, it was possible to show that, as in vertebrates, there is a coupling between depolarisation and secretion¹. One may therefore contemplate the development of a new technique of investigation in neuroendocrinology by electrical stimulation *in vivo* of neurosecretory cells. We were able to provoke by this way a precocious ovarian development in female locusts.

Experiments involving ablation and implantation showed that oocyte maturation and egg laying are under the endocrine control of the PI²⁻⁵ and a pair of endocrine glands, the corpora allata (CA)⁶. We tested two different situations by using the females of a species (*Locusta migratoria*) in which the oocytes develop immediately after metamorphosis (the first batch of eggs is laid after about 18 d) and the females of a species (*Anacridium aegyptum*) subject to an ovarian diapause of 6 months. The opening of a dorsal tegumentary cephalic window enables access to the PI which is superficial. The stimulatory electrode used is a stainless steel wire 30 μ m in diameter, insulated in an epoxy resin. Rectangular pulses of 30 μ A and 1 ms are applied at 5 s⁻¹ for 10 min between the stimulatory electrode

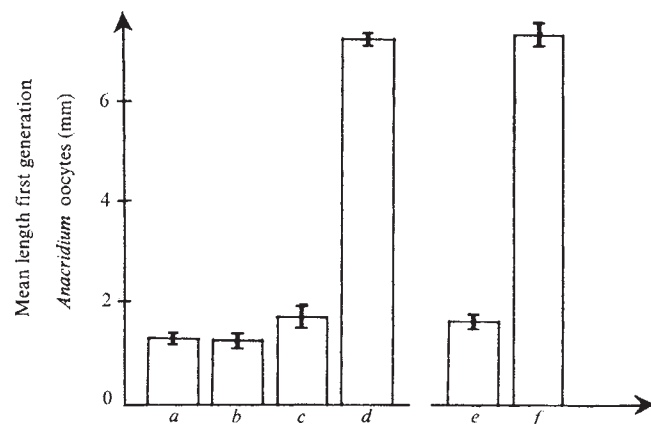


Fig. 2 Mean length (mm) of the first generation of oocytes in *Anacridium* 28 days after various electrical stimulations. *a*, Controls (electrode placed in the PI without passage of current; 14 animals, none died); *b*, stimulation of the ventral nerve cord (14 animals, 2 died); *c*, stimulation of the tritocerebrum (15 animals, 1 died); *d*, stimulation of the PI (13 animals, 2 died, all the survivors laid one batch of eggs, one animal laid two batches); *e*, stimulation of the PI after allatectomy (14 animals, 4 died); *f*, stimulation of the PI after section of the allatocardiac nerves (14 animals, 5 died, 8 animals laid one batch of eggs and 1 contained chorionated eggs ready to be laid).

mals in which the CA have been disconnected (by section of the allatocardiac nerves) experience, after stimulation of the PI, the same growth of the oocytes as that observed in animals in which the CA were not disconnected (Fig. 2*d* and *f*). The mode of action of the PI on the CA is therefore established as humoral rather than nervous.

Our results show unequivocally that the method used provokes a certain release of protocerebral neurosecretion followed by intense activation of oocyte maturation. In the case of *Locusta*, one may thus obtain precocious maturation and in the case of *Anacridium* rupture of the ovarian diapause. This method seems to be more efficacious—at least in the case studied—than the classic techniques of implantation. In effect, to obtain a simple maturation of the ovaries, in *Anacridium*, it is necessary to implant four active PI or four active CA⁵.

Certain general nonspecific stimulations, such as forced activity of the animals or the electrical stimulation of various regions of the brain, may provoke the start of a histologically detectable emptying of the cells of the PI and a very slight development of the oocytes². There is no doubt that the results obtained here are due to a specific stimulation of the PI and not to a general activation: the stimulation of a wide variety of regions of the central nervous system never provoked any maturation of the oocytes.

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M. MOULINS
A. GIRARDIE
J. GIRARDIE

Laboratoires de Biologie Générale
et de Zoologie, Université de Provence,
Centre St-Charles, 13331 Marseille, Cedex 3
France

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Properties of a calcium channel in snail neurones

In most cases where the action potential of nerve cells has been studied the initial inward current has been found to be carried by sodium ions. More recently calcium has been shown to be an important charge carrier in the inward current of vertebrate cardiac muscle, vertebrate smooth muscle and many nerve cell bodies¹. It is therefore of interest to know whether the properties of the calcium conductance mechanism are similar to those which have been described for sodium conductance. An important property of the sodium conductance mechanism is its reduction or 'inactivation' by a conditioning depolarisation prior to an action potential and its increase by conditioning hyperpolarisation.

This activation and inactivation property can be studied in voltage clamp experiments. The membrane potential is displaced from the holding potential (V) to a conditioning potential (V_c) and then to a fixed test potential (V_t) (see Fig. 2). The inward current (I_i) during the test potential is measured and I_i relative to the maximum inward current obtainable with that test pulse (I_i/I_{max}) is plotted against conditioning potential V_c . In squid giant axon Hodgkin and Huxley² showed that this inactivation behaviour was described by the expression:

$$I_i/I_{max} = [1 + \exp(V_h - V_c)/k_h]^{-1} \quad (1)$$

where V_h is the potential at which $I_i/I_{max} = 0.5$ k_h is a shape parameter.

In the *Aplysia* giant neurone Geduldig and Gruener³ found that in Ca-free saline containing Na the relationship between inward current and conditioning potential was similar to that in squid giant axon. In Na-free saline containing Ca ions, however, a hyperpolarising conditioning pulse reduced the inward current. Geduldig and Gruener³ have suggested that there is a fundamental difference between the activation mechanisms for Na and Ca ions in *Aplysia*. In *Helix pomatia* Neher⁴ found a similar reduction of inward current following a hyperpolarising conditioning pulse in normal saline containing both Na and Ca ions but proposed that it was due to activation of a fast transient outward potassium current which subtracted from the inward current. The experiments described here on *Helix aspersa* neurones suggest that the apparent hyperpolarising inactivation of Ca conductance is due to an early outward current which is sensitive to tetraethylammonium (TEA) and calcium ions.

The neurone used in each experiment was identified as Cell A⁵. This cell lies in the right parietal ganglion and shows an inward current in both Ca-free and Na-free salines in voltage clamp experiments. The cell was impaled with two 3 M KCl-filled microelectrodes of resistance 4–6 MΩ and 0.8–1.3 MΩ respectively. A voltage clamp circuit similar to that of Geduldig and Gruener³ was used, giving a voltage rise time of about 100 μs. Normal saline contained NaCl 75 mM, KCl 5 mM, MgCl₂ 15 mM, CaCl₂ 10 mM, Tris-Cl 5 mM, buffered to pH 7.5. In Na-free saline NaCl was replaced by 83mM Tris-Cl. In Ca-free saline CaCl₂ was replaced by MgCl₂. In (Na, Ca)-free saline both substitutions were performed.

In an attempt to eliminate the effect of early outward currents a current-separation procedure was used as follows. A clamping run consisting of a series of different conditioning potentials each followed by the same test potential was performed in the test solution (either Na-free or Ca-free saline). An identical run was then performed in (Na, Ca)-free saline, in which there is no inward current. (There may be an outward current carried by Na and Ca ions but because of their low intracellular concentrations this was small at the test potential used). The current in (Na, Ca)-free saline at a particular time after the beginning of the test pulse was then subtracted from that in the test solution to give the Na or Ca inward current alone. (All runs were interspersed with controls in normal saline.)

Figure 1a shows the results of such an experiment using Ca-free saline as the test solution. I_i/I_o is plotted against the potential of the conditioning pulse. I_o is the inward current with no conditioning pulse and is used instead of I_{max} as the cell is maximally activated at the holding potential. The conditioning potential was 300 ms long which was found to be long enough for maximum effect. The solid line is drawn to equation (1) with $V_h = -27$ mV and $k_h = -4$ (for squid giant axon). Thus the inactivation curve for sodium current is adequately described by the same equation as used for squid giant axon.

Figure 1b shows a similar experiment in Na-free saline (in which the inward current is carried by Ca ions). The solid line

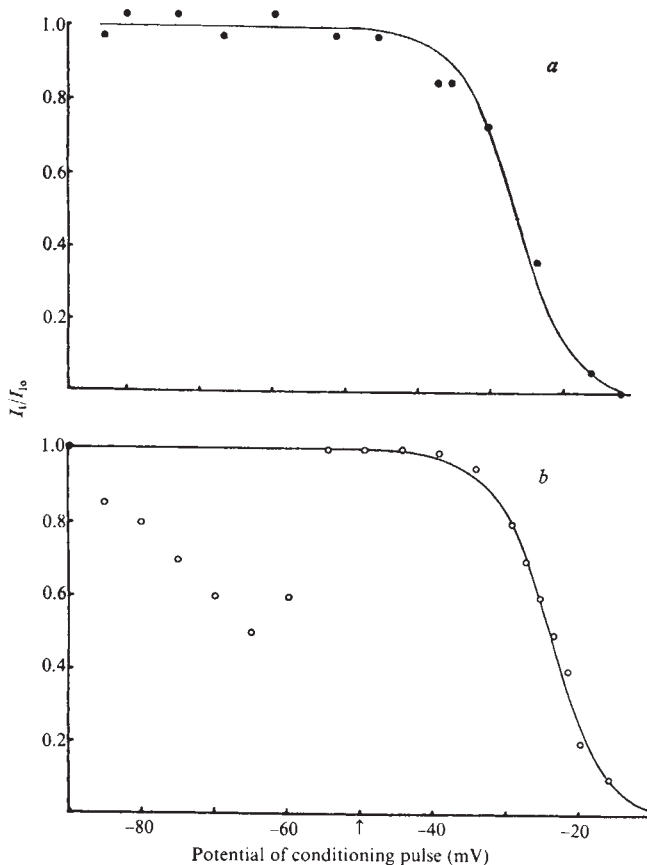


Fig. 1 *a*, Inactivation curve in Ca-free (Na-containing) saline. Ordinate: peak inward current relative to peak inward current with no conditioning pulse (I_i/I_o). Conditioning pulse length 300 ms. Holding potential (V) (arrowed) = -48 mV. Test pulse potential (V_e) = $+14$ mV. Cell 104. Diameter $180 \mu\text{m}$ 22.5°C . Solid line drawn to equation (1) with $V_h = -27$ mV; $k_h = -4$. *b*, Inactivation curve in Na-free (Ca-containing) saline. Conditioning pulse length 500 ms. $V_e = +10$ mV. Cell 139. Diameter $230 \mu\text{m}$ 20°C . Solid line drawn to equation (1) with $V_h = -24$ mV; $k_h = -4$.

is again drawn to equation (1). For depolarising conditioning pulses a good fit is obtained. But with increasing hyperpolarising pulses I_i/I_o falls steeply and then rises more slowly towards unity. Thus there is a considerable deviation from the behaviour described by equation (1). If these results arise from a property of the inactivation system for Ca conductance then this system must be both different from, and more complicated than, that for sodium conductance in *Helix* and squid giant axon. An alternative explanation is that the deviation may arise from the effect of an early outward current which was not eliminated by the current separation procedure. An early outward current activated by hyperpolarising conditioning pulses exists in many molluscs including *Aplysia*, *Helix pomatia* and *Helix aspersa*^{4,6}. If this early outward current was smaller in the (Na, Ca)-free saline than it was in the Na-free saline

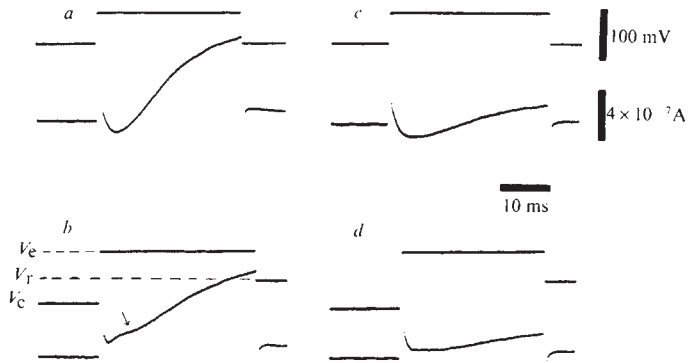


Fig. 2 Voltage clamp records showing effect of TEA. Upper traces voltage, lower traces current. Inward current is shown by a downward deflection. Holding Potential (V) = -48 mV; $V_e = +12$ mV. *a*, no prepulse in Na-free saline; *b*, 500 ms prepulse to -108 mV in Na-free saline (arrow shows early outward current); *c*, no prepulse Na-free saline with 50 mM TEA Cl; *d*, 500 ms prepulse to -108 mV Na-free saline with 50 mM TEA Cl. Cell 168. Diameter $205 \mu\text{m}$ 19°C .

then the amount of outward current subtracted would be too small. This could be, for example, if the early outward current was to some extent Ca-sensitive. There are two ways in which the absence of Ca in the external saline could affect the size of the early outward current. (1) The change in Ca concentration could shift the activation curve of the early outward current along the voltage axis as found for Na and K conductance in squid⁷. (2) The late outward potassium current in *H. aspersa* has a Ca dependent component so it is possible that the early outward current has a similar component⁸.

In order to test whether this deviation from equation (1) did indeed arise from a Ca-sensitive early outward potassium current the effect of 50 mM TEA was tested. This was a sufficient concentration to reduce late K currents. Figure 2 shows current records obtained under voltage clamp in Na-free saline with and without TEA. It can be seen that TEA reduces the late K current and also the early outward current seen with a hyperpolarising prepulse, but does not affect the Ca inward current. Figure 3 shows the effect of TEA on the dip in the inactivation curve in Na-free saline. Open circles are points obtained by subtraction of currents recorded in (Na, Ca)-free saline from currents in Na-free saline—that is, a similar experiment to that shown in Fig. 1*b*. Open triangles are results in which the same subtraction was performed when both salines contained 50 mM TEA-Cl substituted for Tris-Cl. The deviation from equation (1) is greatly reduced and in one experiment was abolished altogether. The TEA has no effect

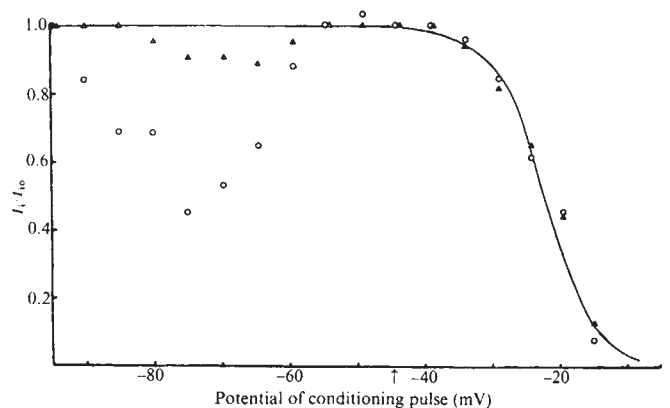


Fig. 3 Effect of TEA on inactivation curve in Na-free saline. $\circ$, Without TEA; $\triangle$, with 50 mM TEA Cl; V (arrowed) = -45 mV; $V_e = +15$ mV. Cell 178. $180 \mu\text{m}$ 21°C . Solid line drawn to equation (1) with $V_h = -23$ mV; $k_h = -4$.

on the normal inactivation by depolarising conditioning pulses.

From the results of these TEA experiments it seems likely that the deviation of Ca inactivation from equation (1) is due as Neher suggested to an early outward potassium current. The current subtraction method of compensating for this current is inadequate, probably because the current is sensitive to calcium. This cannot be shown directly but when K currents are reduced using TEA the deviation from equation (1) is also reduced or abolished. It has recently been shown that hyperpolarising inactivation of Ca current in guinea pig smooth muscle can be blocked by TEA ions⁹. The deviation is also abolished by large hyperpolarising conditioning pulses to potentials more negative than -90 mV probably because the early outward potassium current shifts in time relative to the inward Ca current with increasing prepulse potential.

Thus, it appears that the inactivation behaviour of Ca-conductance in *H. aspersa* is similar to that of the Na-conductance and can be described adequately by equation (1). It is unclear in *H. aspersa* whether the Na and Ca ions pass through the same channel or separate channels. But in vertebrate heart muscle the Ca inward current can be obtained relatively free from other currents in normal saline and does not show hyperpolarising inactivation¹.

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N. B. STANDEN

Zoological Laboratory,
Downing Street,
Cambridge, UK

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Resistance of mitotic B lymphocytes to cytotoxic effects of anti-Ig serum

THE use of antisera with specificity for cell surface antigens of T and B lymphocytes has become an increasingly important tool in cellular immunology, being extensively used both preparatively, and analytically^{1,2}. Very little is known, however, about the possibilities of variation in antibody-induced cytotoxicity during the various stages of the cell cycle of normal T and B lymphocytes. Studies mostly examining histocompatibility or virus-associated antigens on synchronised cell cultures of various tumour cell lines have yielded conflicting findings; in some cases cells seem to be most sensitive to antibody and complement during mitosis and perhaps early G₁³⁻⁵, whereas in others, the target cells have been judged to be insensitive in all phases except G₁^{6,7}.

If, depending on the nature of the lymphocyte and cell surface antigen, there is a similar variation to antibody-induced cytotoxicity during different phases of the cell cycle, this could have clear practical and theoretical implications. In particular, antisera are sometimes used to eliminate or assess cells in antigen-activated lymphoid cell populations which may contain a significant proportion of specifically activated cells, in various stages of the cell cycle, including mitosis. In this communication we

present evidence to show that although mouse B lymphocytes are uniformly killed by a heterologous anti-mouse immunoglobulin serum (anti-Ig) in the presence of complement, B cells in mitosis are resistant to such treatment.

Heterologous anti-Ig sera are being used with increasing frequency to kill B lymphocytes (see for example, refs 8-11); since B cells, in contrast to T cells, contain a dense coat of cell surface Ig (ref. 12), the cytotoxicity of anti-Ig can be selective for B cells after appropriate absorption. The preparation and cytotoxic properties of the anti-Ig used in these experiments is described in Table 1. In agreement with the other results^{9,10}, treatment of spleen cells with the anti-Ig, after it had been absorbed with thymocytes,

Table 1 Cytotoxic effects of rabbit anti-mouse Ig serum plus complement on mouse lymphocytes

Lymphoid cell source	Cytotoxic index
Thymus	2
Thoracic duct	12
Lymph node	21
Bone marrow	37
Spleen	48
Spleen	>95
Spleen†	88
Spleen‡	14
Spleen§	12

The rabbit anti-mouse Ig serum used in these experiments was prepared as follows: 50 ml of neat serum from a large number of CBA mouse donors was precipitated in saturated ammonium sulphate to isolate gamma globulins¹⁸. The precipitate was dissolved in saline and the process repeated twice. The final precipitate was dissolved in 4 ml of saline. Two millilitres of this was mixed with 2 ml of Freund's complete adjuvant (Burroughs Wellcome) and injected intramuscularly into a New Zealand White rabbit. The rabbit was boosted 2 and 4 week later with an intravenous injection of 1 ml of the saline solution containing the dissolved mouse γ globulins. Two weeks later the rabbit was bled, the serum collected and frozen at -20° C in 0.5 ml aliquots. The antiserum was first heat inactivated at 56° C for 30 min. Before use it was absorbed with CBA mouse thymocytes by incubating three volumes of antiserum with one volume of packed washed thymocytes for 60 min at 0° C with shaking every 5 min. The antiserum was tested for cytotoxicity using a two stage microcytotoxicity system¹⁶, in which the first stage was carried out at 0° C so as to prevent capping of cell surface Ig by the anti-Ig¹³. Centrifugations were carried in the cold. Assessment of dead cells was calculated by trypan blue exclusion testing. Normal rabbit serum was used as a control and the results expressed as a cytotoxic index, that is:

$$\frac{\% \text{ dead cells (anti-Ig)} - \% \text{ dead cells (NRS)}}{\% \text{ dead cells (NRS)} \times 10^{-2}}$$

*Anti-Ig not absorbed with thymocytes in this case.

†Cells obtained from adult thymectomised, lethally irradiated, bonemarrow reconstituted mice, 2 months after reconstitution.

‡Anti-Ig absorbed with mouse γ globulins (M γ G) as well as thymocytes. Neat anti-Ig (0.5 ml) was mixed with 0.5 ml of Sepharose beads coated with M γ G by cyanogen bromide activation. The incubation was carried out at 0° C for 1 h. Anti-Ig not absorbed with M γ G and diluted 1:4 as a control gave over 40% kill in this experiment.

§The primary stage of the assay was carried out at 37° C.

inhibited the *in vitro* response to *Escherichia coli* LPS but spared the PHA response (M. J. D. and R. S. K., unpublished observations). The % of cells killed from the various tissue sources is very close to the known corresponding percentage of B cells as assessed by other methods². If the primary stage of the assay was carried out at 37° C instead of 0° C, cytotoxicity was greatly diminished as found by Takahashi *et al.*¹³ which is presumably due to polarisation and loss of cell surface Ig induced by the anti-Ig ('capping') which occurs at 37° but not at 0° C (ref. 14). Absorption of the antiserum with mouse γ -globulin also markedly reduced the cytotoxic activity of the anti-Ig. Before thymus cell absorption, the anti-Ig killed almost all nucleated cells in the spleen and thymus, but after absorption, it was

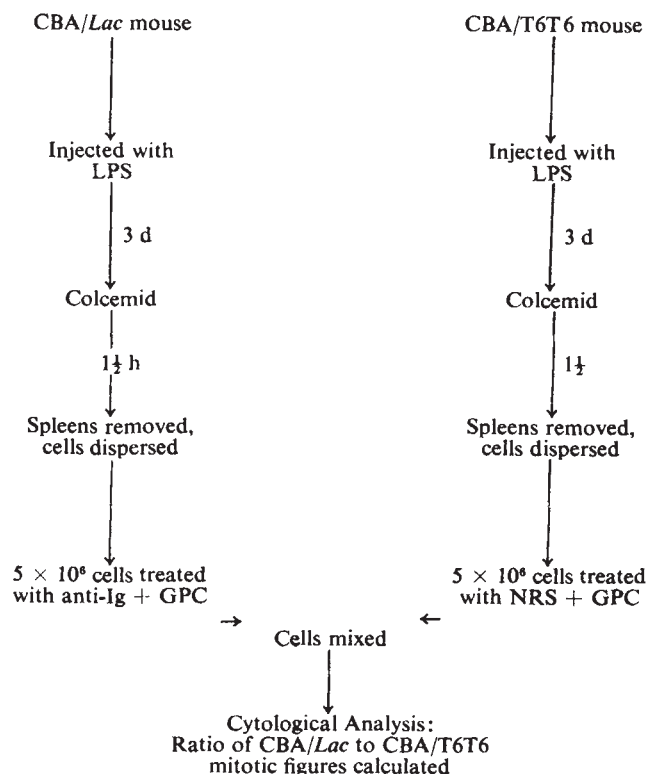


Fig. 1 The assay system¹⁶ used to treat and evaluate the effects of antisera (in this case, anti-Ig) plus GPC on mitotic B lymphocytes. Mitotic T cells are induced by stimulation with oxazolone and removing draining lymph nodes 3 d later¹⁶. Not shown above is the control in which both CBA/Lac and CBA/T6T6 cells are treated with NRS+GPC prior to mixture and cytological analysis. Since the cells are mixed in equal numbers, the ratio of mitotic figures should be roughly 50:50. The effect of the antiserum treatment on the CBA/Lac to CBA/T6T6 mitotic figures in the two different mixtures¹⁶. The percentage of mitotic cells killed was calculated by a cytotoxic index in which the ratio of CBA/Lac to CBA/T6T6 in the antiserum treated mixture is subtracted from the NRS-treated mixture and divided by the latter ratio. The figure is then multiplied by 100. CBA/Lac can be distinguished from CBA/T6T6 mitotic figures by virtue of the fact that the latter contain a tiny pair of marker chromosomes which can be easily visualised when the cells are arrested in metaphase¹⁷. The LPS used was *E. coli* 0:55 LPS (Difco) and the colcemid was purchased from Ciba Labs Ltd. (Horsham, Sussex).

devoid of cytotoxic activity when tested against thymocytes, though still able to kill almost 50% of spleen cells.

The anti-Ig was then examined for its ability to kill B lymphocytes arrested in mitosis. In addition, other antisera were tested such as anti- θ (ref. 1), anti-H2 and anti-MBLA¹⁵. The assay system used has been described in detail¹⁶; it uses chromosome marker techniques¹⁷ and is outlined diagrammatically in Fig. 1.

In Table 2 are recorded the results of experiments in which several types of antisera were tested for their ability to inhibit oxazolone-induced mitotic T cells¹⁶, or LPS-induced mitotic B cells. Anti- θ eliminated 100% of mitotic cells found in the draining lymph nodes of oxazolone-stimulated mice, as did anti-H2 serum. Conversely both anti-MBLA (a marker for B cells¹⁵) and anti-Ig were without such an effect. When LPS-induced mitotic cells were treated, anti-H2 and anti-MBLA were both found to be highly cytotoxic, whereas anti- θ was totally devoid of any such activity. But the anti-Ig was also found incapable of killing these LPS-induced mitotic cells in spite of the fact that 47% of the LPS-immune spleen cells could be killed in the same suspension as assessed by trypan blue cytotoxic testing.

In a parallel experiment, *E. coli* LPS was used as a mitogen to activate spleen B cells *in vitro*¹⁸ and PHA (Burroughs

Wellcome) was used to activate T cells¹⁹ in separate control cultures using methods described previously²⁰. Again anti-Ig was found to possess little ability to eliminate mitotic cells activated *in vitro* by LPS, whereas anti- θ eliminated about 90% of PHA-activated mitotic cells (Table 3).

It may be that the cell surface Ig molecules associated with B cells undergo a density or possibly distributional change²¹ during mitosis such that the cells are no longer capable of being lysed by anti-Ig and GPC. Antigen density on cells is known to play a critical role in complement-mediated cytolytic reactions²² as well as antigen distribution²¹. Buell and Fahey²³ tested Ig-bearing and Ig-producing human lymphoid culture cell lines during different phases of the cell cycle for the presence of Ig by direct immunofluorescence techniques. They found little Ig apparent immediately before, during, and immediately after mitosis; in contrast the G₁ and S phases were highly positive²³. Similar results were obtained by Takahashi *et al.*²⁴.

A factor which should not be overlooked in these experiments is the possible effect of colcemid itself on cell surface Ig: mitotic inhibitors such as colcemid are known to have an effect on lymphocyte receptor mobility²⁵. It should, however, be stressed that anti-Ig+GPC still killed almost 50% of spleen cells from LPS-injected mice which had been given colcemid *in vivo*, and where the assay was carried out in the presence of colcemid (Table 2).

We have also found that treatment of LPS-immune, colcemid treated spleen cells with rabbit anti-Ig followed by a sheep anti-rabbit immunoglobulin serum in the presence of complement was still incapable of killing cells arrested in mitosis (Table 3) (R. S. K., unpublished observations), which suggests that there may be loss of cell surface Ig on the mitotic cells. We are presently studying the cell surface Ig on mitotic B cells using peroxidase staining and electron microscopic

These results indicate that treatment of antigen-activated lymphoid cell populations with anti-Ig plus complement to kill B cells (for example, ref. 11) may spare a small number of specifically activated cells which could partially

Table 2 Cytotoxic effects of various antisera plus complement on non-dividing and on mitotic T and B lymphocytes

Stimulus	Lymphoid source	Antiserum	Cytotoxic index (trypan blue)	% Kill of mitotic lymphocytes
Oxazolone	lymph node	anti- θ *	68	100
Oxazolone	lymph node	anti-H2†	> 95	100
Oxazolone	lymph node	anti-MBLA‡	23	0
Oxazolone	lymph node	anti-Ig	20	0
<i>E. coli</i> LPS	spleen	anti- θ	28	0
<i>E. coli</i> LPS	spleen	anti-H2	> 95	98
<i>E. coli</i> LPS	spleen	anti-MBLA	32	84
<i>E. coli</i> LPS	spleen	anti-Ig	47	4

The assessment of cytotoxic activity on mitotic lymphocytes of the various antisera was carried out as described previously¹⁶ and shown in Fig. 1. Just before mixing the antiserum-treated CBA/Lac cells with the control treated CBA/T6T6 cells, a small aliquot was withdrawn and assayed for the % cells killed by the dye exclusion method. Control treated CBA/Lac cells were also tested and the cytotoxic index calculated. An equivalent aliquot was withdrawn from the CBA/T6T6 cell suspensions to control for cell loss before mixing and cytological analysis. Draining axillary lymph nodes or spleens were removed 3 d after stimulation of the mice. In all cases 250 μ l of neat antiserum was used to treat 5×10^6 nucleated cells except in the case of anti-MBLA, where only 100 μ l was used. This gave a lower cytotoxic index than is usually obtained and may account for only 84% of the LPS activated mitotic cells being killed.

*The anti- θ _{C3H} serum which is specific for T cells⁴ was prepared as described previously¹⁶, and its properties have been described elsewhere¹⁶.

†The anti-H2 serum was made by injecting Balb/C (H2^d) with 20×10^6 spleen cells from CBA (H2^k) donors at weekly intervals for 6 weeks and bleeding the mice 1 week after the last injection.

‡The anti-MBLA serum which is specific for B cells after absorption was made according to the method of Raff *et al.*¹⁵ and absorbed with MyG as well as thymocytes.

Table 3 Cytotoxic effects of anti- θ serum or anti-immunoglobulin serum plus GPC on mitotic lymphocytes activated *in vitro* by PHA or *E. coli* LPS

Experiment	Mitogen	Serum treatment of spleen cells from:		Cytological analysis		Ratio of Lac/T6T6	% Kill by antiserum
		CBA/Lac cultures	CBA/T6T6 cultures	CBA/Lac	CBA/T6T6		
1	PHA	a anti- θ	NMS	6	94	0.064	90.4
		b NMS	NMS	40	60	0.667	
2	PHA	a anti-Ig	NRS	35	65	0.538	— 9.1
		b NRS	NRS	35	67	0.493	
3	LPS	a anti- θ	NMS	33	67	0.493	21.8
		b NMS	NMS	39	61	0.639	
4	LPS	a anti-Ig	NRS	39	61	0.639	8.1
		b NRS	NRS	41	59	0.695	

On day 0, CBA/Lac or CBA/T6T6 spleen cells were incubated with either PHA or LPS in a manner as previously described (1×10^6 cells per culture)²⁰. On the evening of day 2 colcemid was added to each of the cultures and they were collected 16 h later. Using experiment 1 as an example, 5×10^6 PHA activated CBA/Lac spleen cells were incubated with 0.25 ml of neat anti- θ , followed by 0.5 ml of 1:8 GPC. The surviving cells were mixed with 5×10^6 PHA activated CBA/T6T6 spleen cells which had been treated with NMS+GPC and the mixture was then subjected to cytological analysis. In 1a, only six of the mitotic figures counted were CBA/Lac in origin. A control mixture (1b) in which both the CBA/Lac and CBA/T6T6 cells were treated with

NMS+GPC, showed that 40 of 100 mitotic figures were CBA/Lac in origin. The % kill by anti- θ was calculated by subtracting the ratio of CBA/Lac to CBA/T6T6 figures in the anti- θ treated mixture from the ratio found in the NMS mixture, dividing by the ratio found in the NMS mixture

and multiplying by 100, that is $\frac{0.667-0.064}{0.667} \times 100 = 90.4\%$ kill

of PHA activated CBA/Lac mitotic cells by anti- θ + GPC treatment. A similar procedure was done where anti-Ig was used. In these cases 5×10^6 PHA or LPS activated spleen cells were incubated with 0.25 ml of anti-Ig at 0°C for 1 h before the antigen was added.

compromise the use of such antisera in such cases. This in turn suggests a means by which it may be possible to 'negatively' select for (and subsequently isolate) a small number of specifically activated B cells. Treatment of a population of antigen-activated, colcemid-treated, lymphoid cells by anti-Ig plus complement should kill all B cells except those arrested in metaphase, and the majority of these mitotic cells should have specificity for the activating antigen.

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Note added in proof: Preliminary electron microscope studies using immunoperoxidase staining have revealed that mitotic B cells do in fact contain large amounts of cell surface Ig molecules, similar to that found on resting B cells.

R. S. KERBEL
M. J. DOENHOFF

Chester Beatty Research Institute,
Fulham Road, London, SW3 6JB, UK

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Yeast-mycelial conversion induced by N-acetyl-D-glucosamine in *Candida albicans*

DIMORPHISM in fungi is generally defined as a reversible transition from a yeast habit of growth (Y) to a mycelial one (M)¹. In *Candida albicans* Y→M transition can occur rapidly in serum^{2,3}, serum substitutes and other natural⁴⁻⁶ and synthetic media⁷. In a few hours the yeast cell or blastospore forms a germ tube which grows as a true mycelium^{8,9}. Since the cellular form depends on wall construction¹⁰, marked modifications in the organisation of wall components are expected to, and in fact do, occur during morphogenesis. Nevertheless, there is chemical¹¹, cytochemical and ultrastructural evidence^{12,13} that the differences between Y and M walls of *C. albicans* are essentially quantitative, strongly suggesting that hyphal conversion in this yeast is controlled by the modulation of pre-existing enzymatic activities rather than by any new factors.

In view of the usefulness and reliability of germ-tube formation in *C. albicans* as a model of hyphal morphogenesis, it is unfortunate that most of the media so far used are too chemically complex to enable an understanding of the biochemical basis of this phenomenon. We are therefore investigating the minimal requirement for germ-tube formation and show here that acetylglucosamine meets this requirement. We shall also discuss a working hypothesis for a possible role of chitin synthetase in the mycelial conversion of *C. albicans*.

Strain A_{1F} of *C. albicans* (Robin) Berkhout was grown as described elsewhere¹³. A standard inoculum of 0.8×10^8 cells (essentially pure blastospores from the late logarithmic phase of growth) was given to a mixture containing the test substances, at the desired concentrations, dissolved in H₂O or in buffer imidazole-HCl 0.01 M, pH 6.6, 7 (standard), in a

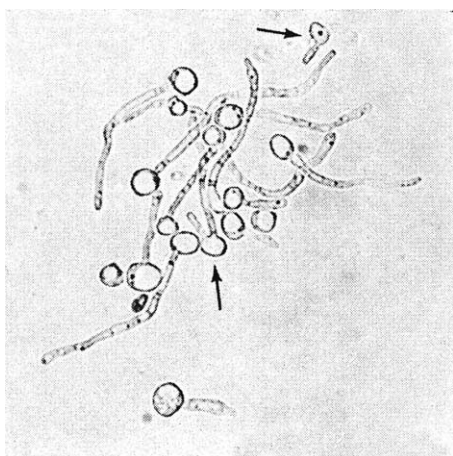


Fig. 1 Germ tubes from blastospores of *C. albicans* after 5 h of incubation in imidazole-HCl buffer, 0.01 M, pH 6.6 containing acetylglucosamine 2.5 mM and MnCl₂ 0.1 mM. The arrows point to cells with two germ tubes ($\times 500$).

final volume of 1 ml. After temperature equilibration, reagents were added and the mixture kept under slight but constant agitation at 37° C. At fixed time intervals, a drop of cell suspension was examined under the light microscope for germ-tube formation, this being recorded as the percentage of elements having at least one recognisable germ tube per 200 elements, regardless of the length of the tube. N-acetyl-D-glucosamine (standard *purissimum*) was obtained from Fluka, Switzerland. All other substances employed were of standard reagent grade.

Owing to the heterogeneity of the initial blastospore suspension, the process of germ-tube formation was essentially asynchronous but in appropriate inducing conditions (see below) after 5 h more than 90% of the cells showed a germ tube of variable length, with many germ

Table 1 Potentiation or inhibition of acetylglucosamine-induced germ-tube formation in *Candida albicans*

Inducer (2.5 mM)	Medium and ions	Other substances	Germination (%)
absent	H ₂ O; Mg ²⁺ or Mn ²⁺ (0.1 mM)	—	3–5
absent	imidazole-HCl Mn ²⁺ (0.1 mM)	—	6–8
present	H ₂ O	—	40–42
present	imidazole-HCl	—	44–47
present	H ₂ O; Mn ²⁺ (0.1 mM)	—	60–65
present	imidazole-HCl Mn ²⁺ (0.1 mM)	—	74–78
absent	imidazole-HCl	cysteine (1 mM)	0
present	imidazole-HCl	cysteine (1 mM)	0

Germination values were taken from two independent determinations, after 4 h incubation at 37° C. Imidazole-HCl buffer was used at pH 6.7 and acetylglucosamine-water measured approximately pH 6.65.

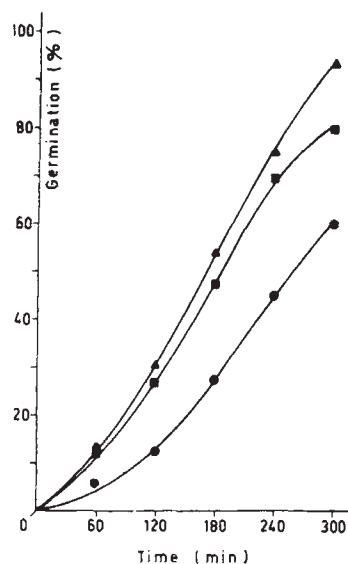


Fig. 2 Time course of germ-tube formation induced by acetylglucosamine 2.5 mM, alone (—●—●—●—), plus MgSO₄ 0.1 mM (—■—■—■—), or plus MnCl₂ 0.1 mM (—▲—▲—▲—). Tests were carried out in imidazole-HCl buffer 0.01 M, pH 6.6, at a temperature of 37° C. Values are means of two independent determinations.

tubes appearing as true hyphae (Fig. 1). The time course of germ-tube formation in various conditions of stimulation with acetylglucosamine is shown in Fig. 2. The germination curve took a sigmoid shape, the maximum rate being recorded between the second and the fourth hour of incubation. The addition of 0.1 mM MgSO₄ or MnCl₂ greatly enhanced the germinating effect of acetylglucosamine (slightly more evident with Mn²⁺). But higher doses of these ions caused no activation but instead a variable degree of inhibition, which is maximal (germination completely abolished) in the presence of 20 mM Mg²⁺ or 25 mM Mn²⁺. Germination did not occur in phosphate Na/K buffer 0.1 M even at high doses of inducer (50 mM) and the same imidazole-HCl buffer was somewhat inhibitory when used at a concentration (0.5 M) higher than that of the standard reaction mixture. Therefore, considering the lack of specificity of these ionic inhibitors, it seems likely that germ-tube formation is sensitive to a high ionic strength. Indeed, high levels of germination can be induced by acetylglucosamine in plain water. Both in water and in imidazole-HCl buffer, the presence of 0.1 mM cysteine completely suppressed the germination (Table 1).

Acetylglucosamine behaves as a strong and rather specific inducer. At 20 μ M it causes, in the presence of 0.1 mM Mn²⁺, 20 to 25% germination after 2 h at 37° C; in this case, however, the phenomenon does not reach completion, even if the mixture is incubated for long periods (12 h). The minimum necessary concentration to achieve full germination falls in the range 0.8–1 mM. Of the analogues tested for their effect on germination (glucose, glucosamine, galactosamine, fructose, glucose-1 phosphate and acetyl-galactosamine) none had any effect until a concentration of 20 mM was reached; at 50 mM, glucosamine and acetyl-galactosamine showed a very slight effect (5 to 8% of germination) of the same order of magnitude as that recorded by incubating blastospores in Mn²⁺-enriched imidazole buffer in the absence of acetylglucosamine (Table 1).

As shown in Fig. 3, the useful ranges of temperature and pH for acetylglucosamine-induced germination in *C. albicans* are rather narrow, the optima falling at around 37° C at pH 6.6. In particular, the germination was markedly low or absent at alkaline pH, at variance with what occurs in serum or other media^{3,6}.

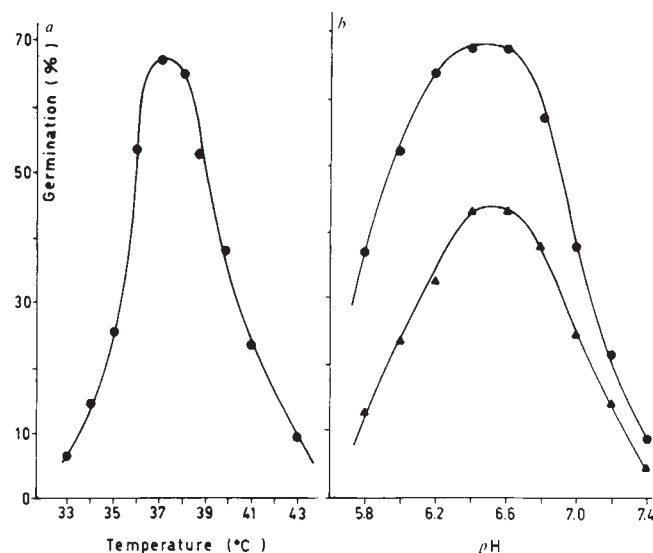


Fig. 3a Temperature dependence of *C. albicans* blastospore germinations induced by acetylglucosamine 2.25 mM in imidazole-HCl 0.01 M, pH 6.6 containing Mn²⁺ 0.1 mM. Effect of pH on germ-tube formation by acetylglucosamine 2.25 mM in imidazole-HCl buffer with (—●—●—●—) or without (—▲—▲—▲—) Mn²⁺ 0.1 mM. The tests were performed at 37°C. Values are means of two independent determinations.

Thus even at very low doses, acetylglucosamine, unlike related carbohydrates, can induce significant germination in the absence of other carbon and nitrogen sources. This, together with consideration of the experimental conditions under which acetylglucosamine induces germ-tube formation in *C. albicans* suggests a specific activating role for acetylglucosamine.

While appropriate experiments must still be performed before an understanding of the mechanism of acetylglucosamine action is reached, we shall consider the possibility that acetylglucosamine directly triggers some key reaction in the dimorphism in *C. albicans*. An obvious candidate for such a role is the chitin synthetase because N-acetyl-D-glucosamine is both the monomer and a specific activator of such an enzyme in yeasts (and other fungi)¹⁴⁻¹⁷.

A controlled activation of chitin synthesis could be a simple way to meet the requirement for increased chitin content of the emerging germ tube and of the hyphal wall¹¹⁻¹³. In *Mucor rouxii*, acetylglucosamine is incorporated, through its UDP derivative, into chitin and it has been observed that chitin synthetase activity is highest at hyphal tip¹⁵. The experimental conditions under which acetylglucosamine induces germ-tube formation in *C. albicans* are comparable to those reported as characteristic of a chitin synthetase assay *in vitro* (pH range, activation by Mg²⁺ and Mn²⁺, specificity)¹⁴. Certainly, as shown in studies on septum formation, the cellular control over chitin synthesis may be highly sophisticated¹⁸ but it is attractive, in line with the results reported above, to think that the levels of free acetylglucosamine in the cell may have a part in controlling overall chitin synthesis and, consequently, germ-tube formation in *C. albicans*. It is also worthwhile to note, in view of the classical concepts on the control of morphogenesis by Nickerson and Falcone^{19,20}, that acetylglucosamine-induced mycelial conversion cannot occur in the presence of an adequate amount of SH compound such as cysteine.

Regardless of these speculations, we have here described an experimental system which is very simple and quite amenable to direct biochemical investigations aimed at an understanding of the biochemical basis of mycelial conversion in *C. albicans*. These investigations may support or

rule out a role for chitin synthetase in this phenomenon.

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N. SIMONETTI

Institute of Microbiology,
University of Camerino,
62032 Camerino (MC), Italy

V. STRIPPOLI
A. CASSONE

Institute of Microbiology,
University of Rome,
00185-Rome, Italy

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Early visual adaptation in goldfish retinal ganglion cells

THE human visual system responds with transient losses of sensitivity when background light levels are suddenly increased or decreased. Crawford¹ measured the early threshold changes in humans by means of a small circular test flash presented before, during and after presentation of a larger diameter adapting light. He found that threshold increased just before the onset of the adapting light, reached a peak shortly after onset, and then decreased during the subsequent 0.5 s. Immediately before the offset of the adapting light, threshold increased slightly, peaked at offset and then returned to its original level. Other investigators have reported essentially the same results under various conditions²⁻⁴. The increase in visual sensitivity during the first few seconds of light adaptation and the peaked loss of sensitivity at the offset of the adapting light are in the wrong direction as predicted by bleaching of the photopigments. Consequently, Baker⁵ suggested that neural factors account for the early change in visual adaptation. Unfortunately, the question of mechanisms involved to account for these rapid changes in visual sensitivity cannot be

answered from psychophysical experiments since the response range cannot be assessed during these periods.

We examined the transient losses of sensitivity at onset and offset of an adapting light in thirteen isolated retinal ganglion cells of the goldfish *Carassius auratus* by extracellular recordings with tungsten microelectrodes. The preparation was an open eye intact procedure developed by Adams⁶. The apparatus, recording procedure and methods have been described previously^{7,8}. All ganglion cells were opponent types; the centre of each cell's receptive field received an inhibitory input from receptors containing a photopigment whose maximum absorption was at 625 nm and excitatory input from receptors containing a photopigment whose $\lambda_{\max}$ absorption was at 530 nm (refs 6, 9). All cells had a central receptive field area of diameter about 1 mm on the retina and a surround larger than 3 mm. The tests and adapting stimuli were focused directly on to the centre of the cell's receptive field under steady photopic background conditions. A 480 nm test light was chosen to stimulate the $\lambda_{\max} = 530$ nm excitatory cone system, while a 570 nm adapting light was chosen because there is an equal quantal absorption at this wavelength by the two goldfish cone photopigments¹⁰.

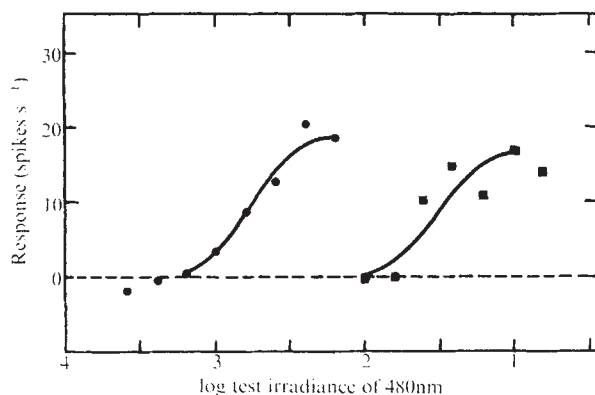


Fig. 1 Intensity response function of the excitatory mechanism of a light adapted opponent ($-R/+G$) cell before and 500 ms after onset of an 0.5 mm adapting light of 570 nm containing 3.6×10^{15} quanta $s^{-1} cm^{-2}$ (abscissa represents log test irradiance of 480 nm, 0 corresponds to $7.2 \times 10^{14} s^{-1} cm^{-2}$). The mean of 10 incremental responses measured in spikes s^{-1} above the spontaneous level during the 0.5 s presentation of the test spot is plotted on the ordinate (dotted line 0 corresponds to the normalised spontaneous level in both figures). ●, Measured at steady photopic levels ($2.2 \mu W cm^{-2}$), spontaneous level being 23 ± 5 spikes s^{-1} . ■, Response function at 0.5 s after onset of the 570 nm adapting light presented at the same photopic background; spontaneous level at this time being $31 \pm$ spikes s^{-1} .

The sensitivity of the excitatory mechanism at various times before, during and after the presentation of adapting lights parallels those seen in human psychophysical experiments, although the time course is longer in this cold blooded vertebrate retina^{7,8}. We measured the intensity response function during the periods when these cells showed a transient loss of sensitivity (Figs 1 and 2). The effect of the onset of the adapting light on a cell's intensity response function is shown in Fig. 1. A smooth curve drawn through the intensity response function at a steady background level illustrates the range of about 1 log unit from threshold to the peak response (in Fig. 1). We refer to threshold-to-peak and not saturation because at higher an intensity level than the peak response the inhibitory 625 nm cone system input reduces the peak response. Adapting light of wavelength longer than 570 nm selectively depresses the sensitivity of the $\lambda_{\max} = 625$ nm cone system and increases the threshold to peak response range⁸. The curve is shifted to overlap the intensity response function measured at 500 ms after the

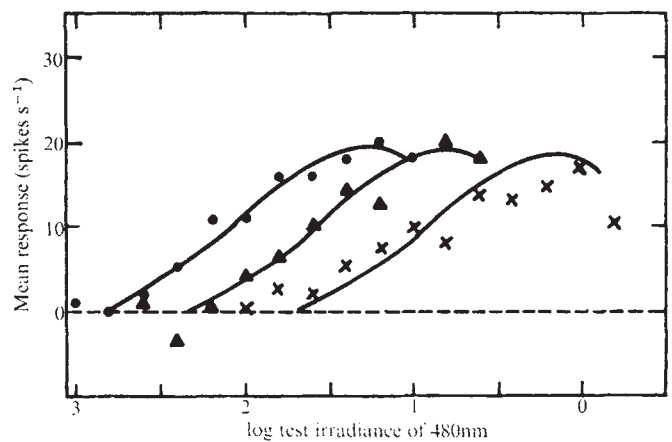


Fig. 2 Intensity response function of the excitatory mechanism of a light adapted opponent ($-R/+G$) cell. ●, Response function at photopic levels of $2.2 \mu W cm^{-2}$, spontaneous level being 9 ± 3 spikes s^{-1} . ▲, Response function at the same photopic level when a 0.5 mm adapting light of 570 nm containing 3.6×10^{15} quanta $s^{-1} cm^{-2}$ has been on for at least 5 min, spontaneous level is 16 ± 4 spikes s^{-1} at this time. X, Response function at photopic levels 0.75 s after the offset of the 0.5 mm adapting spot of 570 nm light, spontaneous level at this time is 30 ± 5 spikes s^{-1} . (Abscissa represents log test irradiance of 480 nm, 0 corresponds to $7.2 \times 10^{14} s^{-1} cm^{-2}$.)

onset of the adapting light. The intensity response function has shifted to the right on the intensity axis, about 1.5 log units, but the range from threshold-to-peak is unaltered. A similar shift in the intensity response function occurs at the offset of the adapting light as illustrated on a different cell in Fig. 2. The intensity response function at a steady background level is shown for comparison. The steady 570 nm adapting light decreased the sensitivity by about 0.5 log units as illustrated by the shift of the intensity response function to the right on the intensity scale. At the offset of the 570 nm adapting light, the sensitivity was further depressed and the range of response is further shifted to the right on the intensity axis. Although it is not shown in Fig. 2, after 3–5 min the response function shifted back to its pre-adapted position. The important generality expressed in these specific examples is that under all conditions of adaptation, transient and steady state, the range of incremental response from threshold to peak remained the same.

The early loss of sensitivity at onset and offset of an adapting light in these experiments was not due to a compression of the response function or a saturation effect at the ganglion cell. The range of light intensity necessary to elicit a threshold to peak response was the same immediately after onset or offset of the adapting list as in steady state conditions. The response function shifted when plotted on a log intensity axis and maintained its general shape and slope. The results show that in these ganglion cells, the operating range shifts across the intensity axis, consistent with a gain change.

The knowledge that gain changes occur in the visual system is neither new nor unexpected, and has been shown in many physiological experiments involving both field and bleaching adaptation^{11–15}. Previously, however, the gain changes, as measured by a cell's response function, were assessed at steady stages of light and dark adaptation where photochemical events are monotonically related to sensitivity. The early threshold events of light and dark adaptation in man are not monotonically related to photochemical events and have been presumed to be limited by neural events. However, in the ganglion cell of the goldfish retina, we have found that the early adaptation events are best described as gain changes and are similar to the change in gain which occurs in the later stages of light adaptation. The signals

from amacrine and horizontal cells show a transient change in the resting potential with the onset and offset of light consistent with the observed shift in the ganglion cell's response function¹⁸. The signal from either of these cells may be involved in the rapid translation and return of the ganglion cell response function across the intensity axis.

Since the early visual sensitivity changes in the human closely parallel those seen in the ganglion cell of the goldfish retina, gain changes may also be responsible for the early transient sensitivity changes observed in humans.

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ARTHUR J. AFANADOR

Division of Optometry,
Indiana University,
Bloomington, Indiana 47401

ANTHONY J. ADAMS

School of Optometry,
University of California,
Berkeley, California 94710

Received January 24; revised April 23, 1974.

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Carcinogenicity of bracken and shikimic acid

SINCE our initial reports of the carcinogenic potential of bracken for rats and mice^{1,2} part of our work has been directed towards the isolation of the causative factor(s). The assays used have included acute toxicity testing in mice (orally and intraperitoneally (i.p.)) and mutagen trials with *Drosophila* and mice, as well as long term cancer induction in rats and mice. The former suffer from the disadvantage of being nonspecific for carcinogens and the latter from the delay of a year or 15 months before results can be assessed. A fraction was isolated, however, which proved positive for acute toxicity, mutagenicity and carcinogenicity in mice and the preliminary molecular formula of $C_7H_8O_4$ was given for the main constituent^{3,4}.

We have now carried the chemical characterisation further and after correction by a low temperature mass spectrum (the addition of a molecule of water to bring the molecular weight to 174 ($C_7H_{10}O_5$), the full analysis gave clear evidence that the isolated compound was shikimic acid (3,4,5 trihydroxy-1-cyclohexene-1-carboxylic acid). The detailed report on the chemical elucidation will be given elsewhere.

At first sight, shikimic acid does not seem to be a likely candidate for a carcinogen, it is widespread in plants⁵, being on the biosynthetic pathway of aromatic ring compounds, and it does not fall readily into the well known groups of chemical

Table 1 Mouse tumours following administration of shikimic acid

Sex	Dose (mg)	Route*	Age at death (weeks)	Neoplasms
M	1	i.p.	70	Glandular stomach advanced grade II
M	5	i.p.	70	Glandular stomach grade II
M	10	i.p.	62	Glandular stomach grade I
M	10	i.p.	62	Glandular stomach early grade I
M	15	i.p.	62	Glandular stomach grade II
M	20	i.p.	58	Reticulum cell B leukaemia
F	20	i.p.	58	Reticulum cell A leukaemia
M	20	i.p.	38	1 Pulmonary adenoma
M	100	ST	34	Lymphocytic leukaemia
				Reticulum cell B leukaemia
				Glandular stomach early grade I
M	10	i.p.	78	—
M	10	i.p.	70	—
M	15	i.p.	62	—
F	20	i.p.	62	—
F	30	i.p.	70	—

* i.p., intraperitoneal; ST, stomach tube.

substances which can induce cancer in experimental animals. Considerable effort was therefore devoted to the minor constituents of the fraction as revealed by chromatography, but fortunately shikimic acid was not entirely neglected and single administrations of the commercially produced acid of tested purity (BDH Ltd) were given to mice. We now have to report the results, to date, of this experiment which are shown in Table 1.

TF1 strain mice (Tucks), averaging 10 weeks of age, were given single i.p. injections of aqueous shikimic acid. Later groups have been dosed orally by stomach tube and the first fatality among these is listed in the table. Out of 14 animals, 9 had cancerous and precancerous lesions. A total of 57 control mice were treated in the same way with various inert materials in aqueous solution and killed after comparable periods of time. It is important that none of these animals had any neoplasms at all, as it indicates that for this strain of mice the spontaneous tumours of old age are not encountered at ages of 15 months or less.

The leukaemias are classified according to the system described by Dunn⁶, and display the variety of classic features including widespread dispersal through the peritoneal and pleural cavities and involvement of organs such as lymph nodes, spleen, liver, and kidney. The reticulum cell A leukaemia was extremely disseminated, with the elongated and round cell types equally represented. One of the reticulum cell B leukaemias had binucleate giant cells and also a greater mixture of plasma cells with the lymphocytes than usual. Grade I neoplasms of the glandular gastric mucosa include those changes which are limited to the mucosa itself, whereas in grade II invasion through the muscularis mucosae into the submucosa has taken place to give the typical 'carcinoma in situ'. In two of the latter, intestinalisation of the tumour epithelium had also taken place.

In the light of these results a mouse test for the production of dominant lethal mutations was conducted according to the method of Bateman⁷, five mature male mice (TF1 strain, averaging 10 weeks old with weights 25–30 g) were used in each test group and ten injected with 0.5 ml of physiological saline for control figures. One group was injected (i.p. with 25 mg of shikimic acid in 0.5 ml of water, which is approximately the LD₅₀ dose (1000 mg kg⁻¹), and a second group had an oral dose administered by stomach tube of 80 mg in 0.2 ml of water. For the 8 successive weeks of the spermatogenic cycle, the males were mated with four virgin females. These were removed at the end of each week and kept until the 12th day from the midweek of their mating, when they were killed, the uterus removed and scored for total implantations, early deaths indicated by decidualomata, and late deaths.

The results are plotted as the percentage of dead implants against weeks from injection (Fig. 1). The percentage, which has

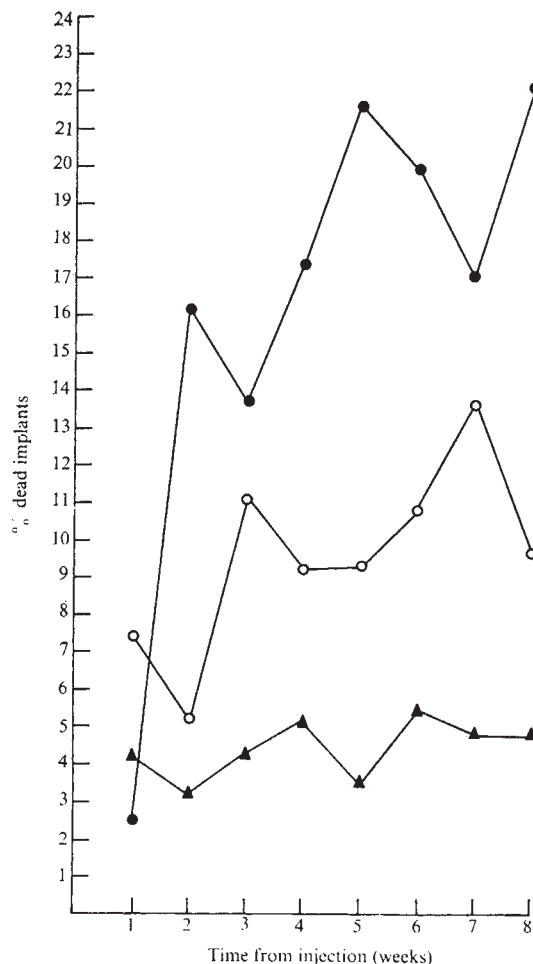


Fig. 1 Dominant lethal mutations induced in mice by commercial shikimic acid. ●, 25 mg (intraperitoneal injection); ○, 80 mg (stomach tube); ▲, control.

also been termed the Mutagenic Index, is derived from the formula:

$$(\text{deciduomata} + \text{late deaths}) / \text{total implants} (\times 100).$$

Each weekly figure is based on the results from 20 females and the numbers not pregnant will decrease the accuracy. It is important that total implants should exceed 100, and in the 16 shikimic groups they averaged 163 (104–210), while the controls averaged 171 (115–237). Taken over the whole 8 weeks, the average control figure was 4.4% (3.2–5.4), whereas the oral shikimic group reached a peak of 13.6 and those injected i.p. were consistently high from the second week onwards, reaching a final maximum of 22.1%.

It is interesting to compare these results with those of Epstein and Shafner⁸ investigating the mutagenic effects in mice of a variety of environmental pollutants, including carcinogens, with an almost identical experimental design. The main difference is that their control mice had an average dominant lethal rate of 1.0% (0–3) while our corresponding figure was 4.4%.

On the other hand, the dosage was of the same order, approximating to LD₅₀ in both cases. On this basis, of the carcinogens listed, only aflatoxin and benzo-a-pyrene reach a level of dead implants of 11% and of all the groups of compounds only METEPA (ref. 8) and related pharmaceuticals reach into the 20% range.

The long term results from previous experiments are now showing clearly that there is a second and different fraction derived from bracken which contains a strong carcinogen and is also capable of inducing the syndrome of acute bovine bracken poisoning (I. A. E., R. S. Jones, D. L. Jones and W. C. Evans, unpublished). That this agent is a more powerful tumour inducer

in mice than shikimic acid is indicated by the fact that single administrations (i.p. and orally) will produce malignant squamous carcinoma of the forestomach as well as the glandular gastric tumours. As well as investigating this fraction, the trials on shikimic acid are being extended to include its effects on other experimental animals and daily small oral dosage as opposed to the single administration tested so far.

We are grateful for financial support from a Cancer Research Campaign grant, the World Health Organisation, Veterinary Division, and a postgraduate research scholarship from Cairo University, Egypt. The low temperature mass spectrum was by courtesy of Drs A. McCormick and C. Baker Harwell.

I. A. EVANS
M. A. OSMAN

Department of Biochemistry and Soil Science,
University College of North Wales,
Bangor, UK

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Phylogenetic origin of xenogeneic recognition

SINCE Metchnikoff (quoted in ref. 1) showed that amoebocytes, the circulating cells in invertebrates, are capable of phagocytosis¹, it has become increasingly apparent that they have various immunological functions. For example, amoebocytes mediate resistance to infection^{2,3} and rejection of xenogeneic grafts^{4,5}.

We have described recognition at the cellular level in the northern sea star, *Asterias vulgaris* (Echinodermata). These animals distinguish between autologous, allogeneic and xenogeneic cell challenge, as evidenced by the development of clumping, and a corresponding decrease in the number of circulating single cells, a phenomenon occurring only in the xenogeneically challenged host. Xenogeneic cells could also be identified in these large multi-cellular clumps⁶.

In addition to host clumping, which is a mechanism of trapping foreign cells, we were interested in determining whether there was a second component to the response, for example, a humoral as well as cellular reaction to foreign antigen. We have now found that a soluble factor which mediates *in vivo* cell aggregation is released by the host in response to xenogeneic but not allogeneic cell challenge.

Common sea stars (*Asterias forbesi*) collected near the Marine Biological Laboratory, Woods Hole, were maintained in tanks containing circulating seawater. Variation in the concentration of unclumped amoebocytes in coelomic fluid fluctuated very little over 60 min (Fig. 1).

Thirty sea stars were inoculated with various cell preparations, both of allogeneic and xenogeneic origin. All 15 xenogeneically challenged animals had decreases in circulation cell counts of greater than 90%. In addition, eight sea stars were evaluated in their initial response: 5 min after challenge when clumping was maximal and the circulating cell count had reached its nadir, the tips of two arms were cut, and the coelomic fluid containing the

Table 1 Effect of cell-free transfer on circulating amoebocyte counts in sea stars

Inoculum	Preinoculum (A) ⁺	Postinoculum (B) [*]	B/A × 100	Time 0 (A) ⁺	Time 5 (B) ⁺	B/A × 100
Seawater	1.3 × 10 ⁶	1.0 × 10 ⁶	77	1.3 × 10 ⁶	1.1 × 10 ⁶	85
Carbon particles	2.3 × 10 ⁶	1.1 × 10 ⁶	48	1.9 × 10 ⁶	4.5 × 10 ⁵	24
Sea star	7.0 × 10 ⁵	4.0 × 10 ⁵	57	1.3 × 10 ⁶	9.5 × 10 ⁵	73
Sea star	1.9 × 10 ⁶	1.7 × 10 ⁶	89	6.0 × 10 ⁵	6.5 × 10 ⁵	108
Sea urchin	2.4 × 10 ⁶	9.0 × 10 ⁴	<1	1.7 × 10 ⁶	3.0 × 10 ⁴	2
Horseshoe crab	1.3 × 10 ⁶	3.0 × 10 ⁴	2	1.3 × 10 ⁶	4.0 × 10 ⁴	3
Horseshoe crab	3.5 × 10 ⁷	1.3 × 10 ⁵	<1	1.7 × 10 ⁶	3.5 × 10 ⁵	21
Human RBC	7.5 × 10 ⁵	2.0 × 10 ⁵	27	1.8 × 10 ⁶	4.0 × 10 ⁴	2

Sea stars were inoculated with 3.0 ml seawater, 1 × 10⁸ carbon particles, 1 × 10⁶ sea urchin amoebocytes, 1 × 10⁶ horseshoe crab amoebocytes, or 1 × 10⁸ human red blood cells. Stars were counted just before inoculation (time 0 (A)) and 5 min after inoculation (time 5 (B)). Percentages of single cells (B/A × 100) were determined. Precounted naive animals were then inoculated with 1.0 ml cell-free supernatant from the challenged sea stars, 5 min counts were obtained, and percentages (B/A × 100) determined.

* Numbers represent single amoebocyte count per ml coelomic fluid.

cells was collected. It was then centrifuged for 20 min at 280g and 1.0 ml of cell-free supernatants (as verified by microscopy) inoculated into a secondary 'naive' recipient. The concentrations of single amoebocytes in the recipient was determined before and at intervals after inoculation.

As Table 1 shows, when either seawater or sea star 'allogeneic' cells were used as challenge inocula, clumping did not occur either in the challenged animal or the passively transferred recipient. Inoculations of sea urchin, horseshoe crab or human peripheral blood cells resulted in a sharp decline in the circulating cell count. Supernatants from positive responders in turn initiated the same response in naive recipients. An example of the response of sea stars treated with cell-free supernatants from either non-reactive or reactive animals is shown in Fig. 2. Only when clumping occurred in the xenogeneically challenged animal was there a corresponding decline in single cell concentration of the passively transferred recipient. Carbon particles induced a moderate clumping response which could be transmitted in the supernatant, indicating that host responsiveness rather than lysis of inoculum was primarily responsible for subsequent *in vivo* clumping.

Prendergast⁷ has isolated a protein (SSF) with a molecular weight of 32,000 from the amoebocytes of the sea star which he has shown mediates clumping in sea stars treated with SSF. It is possible that the factor mediating *in vivo* amoebocyte clumping when transformed to secondary recipients in our system, may cross react with SSF.

The data suggest that recognition in echinoderms is char-

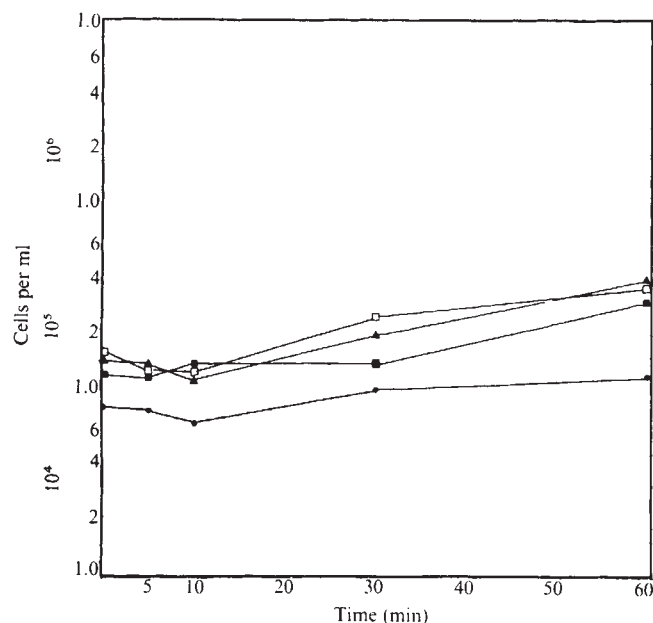


Fig. 1 Single amoebocyte counts per ml coelomic fluid were determined over time in four normal animals.

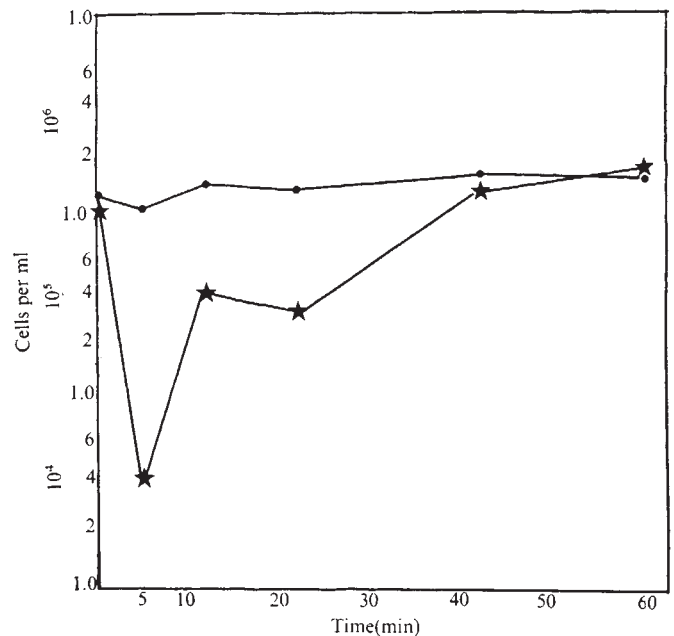


Fig. 2 Sea stars were inoculated with 1.0 ml cell-free supernatants from animals given either 3.0 ml seawater (●) or 1.0 × 10⁶ horseshoe crab amoebocytes (★). Circulating single cell counts were determined over 1 h.

acterised by the following *in vivo* sequence: (1) distinction of xenogeneic antigen; (2) release of activator; (3) clumping, and (4) trapping of xenogeneic cells. The mechanism of host distinction between allogeneic and xenogeneic antigen is unclear. It is becoming apparent, however, that at this stage of phylogeny xenogeneic rather than allogeneic recognition is an intrinsic property of the relatively undifferentiated amoebocyte in contrast to vertebrate and mammalian lymphocytes which respond vigorously to alloantigens.

This work was supported in part by a grant from the National Cancer Institute. Technical assistance by H. Cantor is gratefully acknowledged.

CAROL L. REINISCH

Children's Cancer Research Foundation,
Department of Medicine, Harvard Medical School,
35 Binney Street,
Boston, Massachusetts 02115 and
Marine Biological Laboratory, Woods Hole,
Massachusetts 02543

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¹ Lectures on the comparative pathology of inflammation, (Trench, Tribner and Co., London, 1883).

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³ Bang, F. B., *J. Reticuloendothelial Soc.*, **7**, 161 (1970).

⁴ Hostetter, R., and Cooper, E., *J. Immunol.*, **9**, 384 (1973).

⁵ Ghiradella, H. T., *Bio. Bull.*, **128**, 77 (1965).

⁶ Reinisch, C. L., and Bang, F. B., *Cell. Immunol.*, **2**, 496 (1971).

⁷ Prendergast, R. A., Cole, G. A., and Henney, C. S., *Ann. N.Y. Acad. Sci.* (in the press).

matters arising

Turtle drift

SIR,—It occurs to me that the origin of the peculiar migration pattern of the green turtle, *Chelonia mydas* may be otherwise than suggested by Carr and Coleman¹. They argued that since the early Cretaceous certain turtles have inherited a tendency to swim a particular WNW–ESE path from Brazil to the Ascension Islands, swimming against the prevailing current for about eight weeks. They note, however, that *Chelonia*, which is a herbivorous, frequently seagrass ('turtle grass') eating form, is not recorded before the Miocene. This concurs with my observations² on *Thalassia* (turtle grass) and its associated biota which did not succeed in reaching the tropical Americas until the early Miocene. It seems likely that these seagrasses came from the Indo-Pacific through the southern tip of Africa, and drifted passively across to Brazil from West Africa on the equatorial surface current. Therefore seagrass feeding grounds at least, were situated on the eastern side of the Atlantic first. One might infer from this that the herbivorous turtles followed the seagrass biota westwards across the Atlantic (through the mid-oceanic islands) but returned for the breeding season. If this is so, then the early ancestors of *Chelonia mydas* should be found in Africa or further east.

A second consideration is that the behavioural pattern suggested by Carr and Coleman seems to make little ecological or evolutionary sense (which they have almost admitted). They imply that the home breeding grounds around South America became inexplicably untenable (presumably many millions of years after turtles had become established) so favouring a change in behaviour in these otherwise ultraconservative creatures. The progeny produced by this 'freakish' behaviour were then lucky enough to drift back to the old feeding grounds on the current. Bearing in mind the extremely conservative breeding behaviour of past and present amphibians and primitive reptiles I feel this postulation will need a more thorough explanation.

Would it not be more in keeping with the evidence to postulate the passive drifting of juveniles westwards across the Atlantic (where, after the Palaeogene they would have found plentiful seagrass) followed at breeding

time by a homing instinct that leads them eastwards to the midoceanic islands? The seafloor spreading part of their hypothesis would work equally well.

Yours faithfully,

MARTIN D. BRASIER

Geology Department,
The University,
Whiteknights,
Reading RG6 2AH, UK

¹ Carr, A., and Coleman, P. J., *Nature*, **249**, 128 (1974).

² Brasier, M. D., *Nature*, **243**, 342 (1973).

No radioactive silver detectable in silver–uranium ore

SIR, — Lindner *et al.*¹ have reported finding the radioactive silver isotopes ^{108m}Ag and ^{110m}Ag in silver bars of eastern European origin, and have conjectured that the silver may have been mined using nuclear explosives. Boyle² has suggested that it was more probable that the radioactive silver had been produced by neutrons from (α , n) reactions and spontaneous fission reactions of uranium in the silver ores. In January 1974, I was able to secure a sample of uraniferous silver ore from a mine at Contact Lake, North West Territories, near Port Radium on Great Bear Lake. The 70 g sample was crushed and ground. The resulting powder was assayed using X-ray fluorescence spectroscopy and was found to contain 6.8% Ag and 0.97% U. Fifty grams of the powder was roasted, treated with HF and H₂SO₄, leached with HNO₃ and the extracted silver was precipitated as the chloride. Four grams of AgCl, corresponding to 3 g of Ag, was counted in a defined geometry on a large, high resolution, low background Ge(Li) detector for 25 h. No silver gamma peaks were detected.

The 25 h background of the detector in the region of the 658 keV ^{110m}Ag γ ray was 110 counts. Using the detection limit³ of $4.65\sigma_B$, where σ_B is the standard deviation of the background for paired observations, gives a detection limit of 50 counts in the peak.

Lindner *et al.*¹ found that the silver bullion contained 84 pCi of ^{110m}Ag per gram of silver. If this figure is used to calculate the expected number of counts in the same peak, using a 3 g sample of

silver, a 25 h counting time, 94% peak abundance (ref. 4), 48% isotopic abundance of the parent isotope, ¹⁰⁹Ag (ref. 4), and a measured absolute efficiency of the detector of 2.8% for that energy, then the peak would contain 1.1×10^4 counts. The ratio of the ^{110m}Ag found in the bullion to our detection limit for radiosilver from the Canadian ore is $1.1 \times 10^4/50$, or 220. Therefore it seems that Boyle's suggestion that the radioactive silver found in the bullion by Lindner *et al.* was of natural origin is incorrect.

I thank R. R. Wallace for obtaining the ore sample and G. J. Jarbo for the silver and uranium analyses.

Yours faithfully,

W. F. MERRITT

Biology and Health Physics Division,
Chalk River Nuclear Laboratories,
Atomic Energy of Canada Limited,
Chalk River, Ontario, Canada

¹ Lindner, L., Brinkman, G. A., and Schimmel, A., *Nature*, **240**, 463 (1972).

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³ Adams, F., and Dams, R., *Applied Gamma-Ray Spectrometry*, 233 (Pergamon Press, Oxford, 1970).

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ABO matching in kidney graft survival

SIR,—Joysey *et al.*¹ reported on kidney graft survival in Cambridge with particular reference to the ABO group of the recipient. The data from Birmingham confirm these findings and allow a further breakdown by ABO status of the donor.

A system of computer documentation of transplantation and serological data is being used, which includes the display of survival on a graphical plotter. The data are presented in a direct form, showing the fraction of grafts surviving, rather than as 'actuarial' curves. The graphs, therefore, may show irregularities instead of a smooth descending curve. The advantages and disadvantages of various methods of presenting survival data will be discussed elsewhere.

Excluding second and third grafts, 161 patients were transplanted between May 1968 and January 1974 with cadaveric kidneys. Figure 1a shows the survival information in intervals of 3

months over a 4-yr period. All graft failures are included. Survival in group O and non-group O recipients shows the same difference as in the Cambridge data, that is that grafts survive better in group O patients. At 1 yr a 2×2 test shows formal significance, $\chi^2 = 6.49$, $P = 0.02-0.01$; however this test was only made after looking at the graphical output. The group A patients were divided according to whether they received group O or group A donor kidneys, Fig. 1b. There seems to be no initial difference in survival, but the numbers are small.

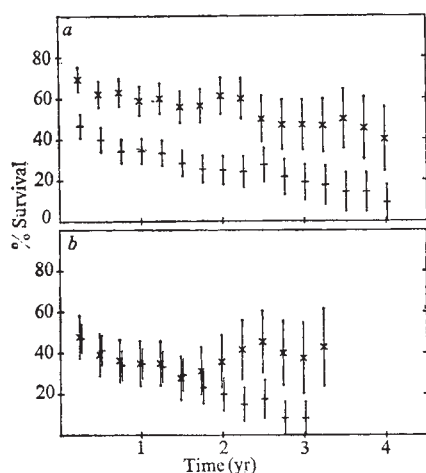


Fig. 1 Survival of kidney grafts in Birmingham. a, Survival in group O (x) and group A (+) recipients. b, Subdivision of group A recipients according to whether they received group O (x) or group A (+) kidneys. There were 62 group O recipients; the 75 group A recipients received 51 group A and 23 group O kidneys, and one of unknown blood group. Vertical bars represent standard error.

Only group O recipients are guaranteed an identical ABO match. If a mismatch for the product of the O gene is not acceptable, using the genotype frequencies for southern England from Race and Sanger², this will only account for 14.4% of A to A transplants and 17.4% of O to A transplants because the majority of group As will be heterozygous AO. Is this, together with an allowance for A_1 and A_2 mismatching, enough to account for the observed discrepancy in graft survival between group O and group A recipients? If ABO genotype is important, then without A_1 and A_2 grouping and matching, only 44% of A to A grafts will be genotypically identical.

I am indebted to Professor J. H. Edwards and Mrs Karen Glenn for developing the system of computer documentation.

I thank Mr A. D. Barnes for making the survival information available and the Kidney Research Fund for financial

assistance with the analysis.

Yours faithfully,

PAULINE MACKINTOSH

Regional Transfusion Service,
Vincent Drive,
Birmingham B15 2SG, UK

¹ Joysey, V. C., Roger, J. H., Evans, D. B., and Herbertson, B. M., *Nature*, **246**, 163 (1973).

² Race, R. R., and Sanger, R., *Blood Groups in Man* (Blackwell Scientific, Oxford and Edinburgh, 1968).

Spittlebug morph mimics avian excrement

STR.—Most colour forms^{1,2} of the spittlebug, *Philaenus spumarius*, are cryptic, but the *marginella* morph has a black body outlined by white. Thompson³ has proposed that this phenotype represents an example of escape warning coloration, suggesting that experienced predators ignore *marginella* individuals because they remember previous "frustrating encounters" with *marginella* more vividly than similar encounters with cryptic morphs. The logic of this argument is tenuous. It seems equally likely that predators should chase *marginella* more actively because of an equally vivid recollection of successful encounters. Moreover, if the phenotype was a warning pattern its fitness should increase with increased frequency, for a greater proportion of experienced predators would be encountered. Both cryptic and *marginella* forms must be present for such conditioning, but this is assured even when the *marginella* allele is fixed, for only the females are brightly coloured, males are cryptic². On this basis fixation of the *marginella* allele should occur, yet in natural populations its frequency remains low.

Patterns of black and white have functions other than predator warning; among small arthropods such patterns are frequently associated with excrement mimicry⁴. Indeed the *marginella* pattern, coupled with the elongate shape of *P. spumarius*, produces a convincing resemblance to a bird dropping. Ignoring possible complications, such as the occurrence of other excrement mimics or changes in the grouped abundance of the cryptic morphs, *marginella* frequency should be intimately related to model abundance. A uniform distribution of bird droppings could, for instance, be the cause of the relatively constant frequency of the *marginella* morph in the Great Lakes area of North America².

The frequency-dependent fitness of mimetic forms can lead to sex limitation when the timing of predation differs between sexes. For example, if predation is postreproductive in males, but pre-reproductive in females, suppression of male mimicry allows population size to

increase while not affecting male fitness. This situation is closely approximated in *P. spumarius* as mating occurs in the early summer while egg deposition is delayed until autumn⁵. It is not surprising then, that modifiers have accumulated to restrict the *marginella* pattern to females.

In conclusion there seems little reason to suspect that the *marginella* phenotype has any warning significance; rather it mimics avian excrement in a situation analogous to classical Batesian mimicry.

Yours faithfully,

PAUL HEBERT

School of Biological Sciences,
University of Sydney,
Sydney, NSW 2006, Australia

¹ Weaver, C. R., and King, D. R., *Ohio Agr. Exp. Sta. Res. Bull.* **741**, 1 (1944).

² Thompson, V., and Halkka, O., *Am. Midl. Nat.*, **89**, 348 (1973).

³ Thompson, V., *Nature*, **242**, 126 (1973).

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MR THOMPSON REPLIES: I believe these objections to my proposal are not well founded. Cryptic coloration of all forms is not necessary for the maintenance of an apostatic polymorphism. This can be demonstrated quantitatively by a simple extension of the model of Clarke and O'Donald¹ for frequency-dependent selection at one locus with dominance. Let A represent a dominant allele for conspicuous coloration with frequency $1-q$ and A^1 a recessive allele for more cryptic coloration with frequency q . Let t and t_1 represent factors relating the frequency of each phenotype to its selective value, and let I be a standard fitness.

Genotypes	AA and AA^1	A^1A^1
Frequency after random mating	$1-q^2$	q^2
Selective value:		
Case 1	$I-t(1-q^2)$	$I-t_1q^2$
Case 2	$I-t(1-q^2)+w(1-q^2)$	$I-t_1q^2$

At equilibrium the selective values of the two phenotypes must be equal and for this example (Case 1) it can be shown that there is a non trivial stable equilibrium for the two alleles at $\hat{q} = \sqrt{t/(t+t_1)}$ provided that t and t_1 are both positive (each colour form becomes less fit as it becomes more common). Both forms will be maintained in the population with the more conspicuous form at lower frequency.

Furthermore, an allele determining a conspicuous pattern which is also a warning pattern will not necessarily increase to fixation, even though the warning pattern *per se* confers greater fitness as its frequency increases. Let w represent the relation between the frequency of the conspicuous phenotype and that part of its selective value determined by its function as a warning

pattern, with t and t_1 again representing apostatic relations (Case 2). In this case there is a non trivial stable equilibrium at $\hat{q} = \sqrt{[(t-w)/(t-w+t_1)]}$ so that when $t > w$ the tendency of the conspicuous warning pattern to increase will be held in check by apostatic selection. Though this example probably underestimates the complexity of the predator-prey relations in question it does demonstrate that there is no *a priori* reason to reject the hypothesis that *marginella* is a warning pattern maintained in polymorphic condition with more cryptic forms by apostatic selection.

Dr Hebert's counter proposal is difficult to accept. The *marginella* pattern of black on white is too sharp and the black on most specimens too unbroken to reasonably suggest a resemblance to bird excreta. But, as Hutchinson² has noted, the colour form *trilineata* may vaguely resemble grass seeds and I do not rule out protective resemblance as a factor in *Philaenus spumarius* polymorphism.

I thank Dr L. Van Valen for comments. University of Chicago, Illinois 60637

¹ Clarke, B., and O'Donald, P., *Heredity*, **19**, 201 (1964).

² Hutchinson, G. E., *Entomologist's mon. Mag.*, **99**, 175 (1963).

Genetic control of natural resistance to *Leishmania donovani*

SIR,—In studies of infections in mice with *Leishmania donovani*, an intracellular parasite, (in preparation) several observations are relevant to the recent letter¹ on mouse susceptibility to *Salmonella* showing that seven mouse strains fell into two resistance categories.

Colleagues and I have found that the growth rate of *L. donovani* (Ethiopian strain L82) populations in mice varied greatly and examined the acute phase, the first 2 weeks after infection, in detail. Amastigotes from hamster spleen, cleaned by differential centrifugation, were injected intravenously into three mice from each of 25 inbred strains and five F_1

hybrids. Parasite numbers at day 15 in the liver were estimated by Stauber's method^{2,3}. The ratio of parasites to liver cells was determined on liver imprints and multiplied by the liver weight in mg to give a reproducible index of parasite load in LDU (Leishman-Donovan units).

The mean liver parasite load on day 1 of 30.5 LDU did not differ between strains but at day 15 inbred mice fell into two distinct categories which did not overlap. Twelve highly susceptible strains showed about a hundred-fold multiplication whereas the remaining 13 strains were resistant with less than eight-fold increase. No intermediates were seen except for an F_1 cross between a susceptible and a resistant strain which was of intermediate susceptibility. The observation suggested a rather simple genetic control of resistance. Our series included the seven strains studied by Plant and Glynn¹ and the results are in Table 1. There is precise correspondence between resistance to *S. typhimurium* and to *L. donovani*.

In breeding experiments resistant C3H mice were crossed with susceptible NMRI. The F_1 progeny were both selfed and back-crossed to each parental strain. Mice were infected as before. Liver counts from mice killed after 2 weeks are summarised in Fig. 1. Susceptible mice reappear as a distinct group clearly separable from a wider group corresponding to resistant and F_1 categories. The proportion of susceptible mice does not differ significantly from one quarter in the F_2 and one half in the back cross to susceptible parents. Also, the observed distribution of other counts fits closely to the predicted distribution on Mendelian expectations using independent estimates of the means and variances of counts in F_1 and resistant mice.

This is strong evidence for control of *L. donovani* growth in the mouse liver by a single gene or tight linkage group. The coincidence of response pattern to *L. donovani* and *S. typhimurium* has a 1:64 probability of having arisen by chance as there are concordant results for six separate mouse strains (C57BL and B10.D2 are genetically very similar). It is therefore possible that we may be

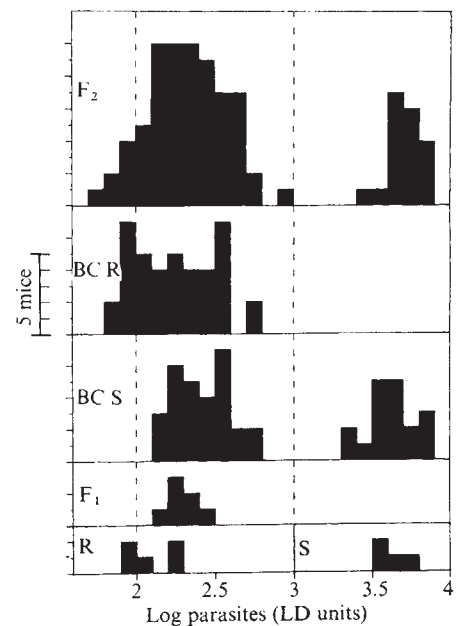


Fig. 1 Histogram of liver parasite burdens, 14–16 d after infection with 10^7 *L. donovani*, from 16th passage, October 1972. Mice derived from crossing susceptible (S) NMRI and resistant (R) C3H strains. Results from F_2 back-crosses (BC) to S and to R, F_1 , and parental strains are shown. Parasite counts have been logarithmically transformed.

dealing with the same genetic mechanism

There is no evidence from our 25 strains that any *H-2* alleles correspond with acute phase resistance or susceptibility, nor have attempts to map the gene (provisionally name *Lsh*, leishmaniasis resistance) given support for any linkage to *Ir* or *H-2* loci (D. J. B. and B. A. Taylor, in preparation). Results so far do not suggest any direct relation of acute leishmaniasis susceptibility to *Ir*1, *H-2* or the ability to mount an acquired immunological response.

I thank Jean Kirkley for technical assistance and the Royal Society and Wellcome Trust for financial support.

Yours faithfully,

DAVID J. BRADLEY

Sir William Dunn School of Pathology,
South Parks Road,
Oxford OX1 3RE, UK

Table 1 Parasite numbers in mouse livers

Mouse strain	Mean count (LDU)	Mean log count* ($\pm$ s.e.)	Parasite increase (day 15 count / day 1 count)	Susceptibility <i>Leishmania</i>	<i>Salmonella</i> †
BALB/c	3,438	3.534 ± 0.030	115.1	S	S
C57BL	3,697	3.565 ± 0.035	123.3	S	S
B10 D2 new	6,410	3.793 ± 0.078	208.4	S	S
DBA/2	94	1.967 ± 0.044	3.1	R	R
C3H/He	165	2.216 ± 0.030	5.5	R	R
A	149	2.170 ± 0.030	5.0	R	R
CBA/Ce	125	2.095 ± 0.040	4.2	R	R

* Here the counts in LDU have been logarithmically transformed which normalises the distribution and renders the variance independent of the mean.

† *Salmonella typhimurium* susceptibility from ref. 1 for comparison.

Parasite numbers were measured 15 d after intravenous infection with 10^7 amastigotes of *Leishmania donovani* derived from hamster spleen, 14th passage, May 1972. Three mice of each strain used.

DR PLANT AND PROFESSOR GLYNN REPLY: Bradley's observation that in strains of inbred mice resistance to infection with *Leishmania donovani* is either very high or very low and corresponds to the level of resistance to *Salmonella typhimurium* found by us in six of the same strains reinforces the idea that resistance is controlled by only one or a few closely linked genes.

Like him, we do not find in further breeding experiments that resistance is invariably associated with a particular *H*-2 type. But because of our results with delayed hypersensitivity reactions as well as on general grounds we do believe that some sort of enhanced immune response is involved. Both *S. typhimurium* and *L. donovani* are intracellular parasites and cellular immunity is the most important defence mechanism in both. It is unlikely though not impossible that there is a significant protective antigen common to *S. typhimurium* and *L. donovani*, though it is also unlikely that anyone has actually looked for one. An immune response gene controlling responses to several unrelated antigens would explain the results.

Department of Bacteriology,
St Mary's Hospital Medical School,
London, UK

¹ Plant, J., and Glynn, A. A., *Nature*, **248** 345-347 (1974).

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Area postrema and blood pressure

SIR,—Ylitalo *et al.*¹ based their suggestion that the area postrema is a control centre of blood pressure on experiments in which a maintained rise in the level of blood pressure, which was also more labile than normal, followed destruction of the area postrema in rats. As it is well known, however, that such effects are produced by section of the buffer nerves², the possibility must be considered that the baroreceptor pathway could have been interrupted where the sinus and aortic nerves terminate in the medulla in the nucleus of the tractus solitarius^{3,4}, which lies immediately adjacent to the area postrema.

We suggest, therefore, that a simpler explanation of the results¹ is that the damage caused by thermocoagulation of the area postrema had spread the fraction of a millimetre necessary to involve the nucleus of the tractus solitarius. In the absence of evidence that the baroreceptor pathway had been spared, it is not justified to put forward the suggestion that

the area postrema acts as a special blood pressure regulating centre.

Yours faithfully,

S. M. HILTON
R. M. MCALLEN
K. M. SPYER

Department of Physiology,
The Medical School,
The University of Birmingham,
Birmingham B15 2TJ, UK

¹ Ylitalo, P., Karppanen, H., and Paasonen, M. K., *Nature*, **247**, 58-59 (1974).

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On fighting strategies in animal combat

SIR,—The article¹ by Maynard Smith and Price is unfortunately based on a number of unwarranted assumptions, and on an inadequate literature research. It perpetuates an old ethological myth that animals fight so as not to injure each other, or refuse to strike 'foul blows' and, presumably, kill each other. The authors assumed that there were such categories as 'conventional' and 'dangerous' in animal conflict, that opponents in combat retreat when injured, and that opponents retain no memory of past contests. None of these assumptions can be regarded as valid. They were not aware of the published field studies primarily of large mammals which have shown not only how dangerous combat is, but, more importantly, have also led to new theories of explaining aggressive behaviour on the basis of individual selection²⁻¹⁰.

Two authors at least^{2,3,11} have developed the concept that combat can be understood as an interplay of defensive and offensive behaviour. This is a simple point but one missed previously, and one that leads to the conclusion that animals need not rely on altruistic impulses in their opponents to escape injury, but rely on their abilities to block, evade or frustrate attacks. First, the inhibition against engagement in overt aggression in species with excellent weapons but poor morphological or behavioural defences can be explained by the principle of retaliation^{2,3}. This explanation assumes that an animal will attack a conspecific if it experiences severe pain, an assumption amply verified^{12,13}. Second, it assumes that even dangerously armed species (excepting humans) usually cannot kill an opponent outright and thus escape retaliation. This second assumption is entirely in line with data

from diverse field studies on carnivores and ungulates^{2-9,14}.

The authors also become victims of one study of mule deer which can be faulted for inadequate observations. Linsdale and Tomich¹⁵ in their work apparently failed to see a fight between mule deer bucks and confused the common sparring matches with fighting. Sparring matches are performed by bucks of unequal size or dominance rank; they are initiated by the subordinate buck and terminated by him; they are long lasting with many engagements and have antler wrestling as their principal behavioural component. Fights are exceedingly rare, occur between matched bucks, and differ strikingly in their execution from sparring matches; moreover the victor chases and attempts to gore the vanquished. There are no 'winners' or 'losers' in sparring matches. Severe wounding does occur in mule deer, usually on smaller bucks unable to withdraw from onrushing dominants which guard females. Flanks, shoulders, haunches and faces are pierced; the rate of visible wounding is about 10% yr⁻¹ among bucks exceeding 1.5 yr of age. I shall report in detail on this in the near future.

Yours faithfully,

V. GEIST

Faculty of Environmental Design,
The University of Calgary,
Calgary, Alberta, Canada, T2N 1N4

¹ Maynard Smith, J., and Price, G. R., *Nature*, **246**, 15 (1973).

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book reviews

Physicist on the pyramids

The Riddle of the Pyramids. By Kurt Mendelssohn. Pp. 224. (Thames and Hudson, May 1974.) £3.50.

DR KURT MENDELSSOHN, the noted cryogenist, has in this book turned his attention to the unrelated subject of Egyptology, in which he confesses an intense amateur interest, and in particular to the riddle presented by the pyramids. Students of the subject may well heave a sigh at the addition of yet another title to the immense literature on Egyptian pyramids which has been written since the days of Herodotus. They may also view with misgiving the entry of a physicist into this unfamiliar field, bearing in mind the intervention of an earlier scientist, Piazzi Smith, the Astronomer Royal for Scotland, who in 1877 produced in his work *Our Inheritance in the Great Pyramid* a source of endless inspiration to the obscurantists who caper on the fringes of the subject. Such fears may immediately be set at rest: Dr Mendelssohn has a unique contribution to make which deserves very careful consideration by the experts as well as the attention of the general reader to whom this book is primarily addressed.

What the author sets out to do is to produce some rational explanation of why such an immense effort was made in raising the great stone pyramids of Egypt over four and a half thousand years ago. The usual explanation advanced by Egyptologists is that such edifices were the tombs of kings who were regarded as gods incarnate, and who according to the religious ideas of the age had to be buried in or under them to ensure the prosperous continuation of the divine government of Egypt. Dr Mendelssohn finds this an inadequate explanation. Some at least of the royal tombs of the Pyramid Age were cenotaphs and what he has to say about this aspect of the problem (pages 74-77) is well worth pondering. He admits that the pyramids served as royal mausolea, not necessarily tombs, but he considers that their funerary function was not the only purpose of their construction, or indeed even the main one.

The point of departure of his enquiry is a discussion of the constructional problems presented by the large stone pyramids at Meidum, Dahshur and Giza. He has already formulated most of his ideas in recent papers in the scientific journals, but in this book

he is able to expand them significantly. The step pyramid which raises its impressive tower above extensive mounds of debris on the desert verges at Meidum was evidently completed by Sneferu, the first king of the fourth Dynasty in about 2600 BC, by adding a cladding of fine limestone, thus converting it to the first true pyramid in Egypt. All the archaeologists who have excavated this monument have agreed that the loss of the outer casing was due to the subsequent operation of stone thieves who used the pyramid as a quarry. Dr Mendelssohn will not accept this finding and has produced impressive testimony to show that the Meidum pyramid collapsed during its final building because its structure had a number of inherent weaknesses. He draws a comparison with the Aberfan disaster; and certainly the aerial view of the pyramid given in his plate 23 clearly shows the plastic flow of the ruined mantle around its nucleus. The shearing-off of this outer covering by such a catastrophe also explains satisfactorily certain architectural features evident in the remaining core and the extraordinarily high mounds of rubble at its base. There are few Egyptologists who will wish to challenge these arguments of Dr Mendelssohn, and pending any final discoveries that may be made by the complete excavation of the pyramid, we may regard his explanation as the most plausible that exists.

The author then proceeds to discuss the effect of this disaster on the design of subsequent pyramids and tries to show that it was responsible for the abrupt change of angle in the Bent Pyramid at Dahshur and for the character of its mantle. He also argues that it promoted improvements in the design of the North Pyramid at Dahshur and the three giants at Giza. If his arguments be accepted, some revision of our views about pyramid building is required since it is clear that two or more great pyramids were being built at the same time during the reign of Sneferu at least, and the implications of all this form the second and more provocative and perhaps more interesting part of the book.

In essence the author's argument is that the consecutive construction of pyramids, one during each reign, is an economic and organisational impossibility. Owing to their immense size the building of pyramids on the scale undertaken in the Fourth Dynasty had become an activity in its own right

demanding its own economic rules. It was the pyramid and not the pharaoh that ruled Egypt; and new pyramids had to be raised whether a pharaoh was ready for burial or not. The erection by the genius of Imhotep of the first great stone building in Egypt, the Step Pyramid of King Djoser in about 2680 BC, began a movement which escalated into a self-sustaining process that affected the seasonal employment of a vast army of workers whose communal feeding, clothing and upkeep for several months during each year must have completely revolutionised the pattern of life for the whole country. A central administration became responsible for the livelihood of a great proportion of the rural and artisan population in place of local councils and village elders. In short, the immense communal activity of building the pyramids created the cohesive Egyptian state with its cohorts of civil servants, and could not be abandoned though it was subsequently modified.

This brief analysis may not do full justice to the author's thesis which is persuasively argued with analogies to the later Mexican pyramids and modern state enterprises. Of course not all the interpretations will command the approval of Egyptologists who form a notoriously sceptical body of opinion; nevertheless we should be grateful to Dr Mendelssohn for obliging us to rethink the received view of the Pyramid Age. One of his readers, at least, will be unable to look again at the monuments of Meidum, Dahshur, Sakkarah and Giza in quite the same old way.

CYRIL ALDRED

Making electricity

Thermionic Energy Conversion. Vol. 1: Processes and Devices. By G. N. Hatsopoulos and E. P. Gyftopoulos. Pp. xi+265. (MIT: Cambridge, Mass. and London, 1973.) \$17.95; £9.00.

THE increasing interest in alternate energy systems makes the appearance of this book apposite; unfortunately like *The Times* it is wise after the event. The method of converting heat to electrical energy described requires thermal sink temperatures high by comparison to room temperature thus making the method suitable in principle for space use. The buildup of the US space programme thus corresponded to a time of intense activity in this field: the rundown of space activity has led to the demise of thermionic energy

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Grassland Ecology and Wildlife Management

E. DUFFEY, M. G. MORRIS, J. SHEAIL,
L. K. WARD, D. A. WELLS and T. C. E. WELLSJuly 1974: 0 412 12290 1: 304 pages:
tone and line illustrations: hardback:
£5.40

This book describes the distribution and ecology of lowland grasslands in Britain with special reference to their flora and fauna, history, and management for wildlife conservation. The maintenance and manipulation of grasslands for agricultural, scientific, conservation and recreational purposes requires an extensive knowledge of the responses of plants and animals to different treatments and disturbance factors. The book examines these in relation to the range of variation in lowland grassland ecosystems and to the known land-use history.

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Further details of these and related titles, together with a list of stockists, are available from the publishers on request.

conversion. It is therefore difficult to see for whom this book was written; without device requirements it will be of little use to the engineer; it will infuriate the physicist.

Volume 1 is intended to summarise the field and present ideas which will not be changed by subsequent developments. Unfortunately the analogues and the presentation of physical principles do not bring out the excitement of understanding and therefore make it worth undergraduate study; the continued reliance on the statement "a more detailed presentation appears in volume 2" means that the physicist without volume 2 will give up in disgust.

The four chapters of this book present an introduction to thermionic converters, an analysis of ideal performance, a description of vacuum converters and finally a chapter on vapour filled thermionic converters. In what is clearly intended to be a definitive book the brevity of the historical review in chapter 1 is surprising, but most of the various types of converter prior to 1966 are well illustrated. The book has, however, clearly been some time in preparation and converters developed after 1966 are not mentioned. The book is lavishly illustrated, so that the relevant text is often several pages away; it is, however, reasonably indexed, gives a large number of references and contains a useful set of tables and curves. The latter when used in conjunction with the step-by-step instructions given in chapters 3 and 4 give reasonable approximations to observed device characteristics and thus form one of the most useful parts of this volume.

In view of the coherence of presentation suggested by the authors for the complete book, it is perhaps unfair to judge this volume in isolation; however without volume 2 it does not seem worth £9.00.

A. W. PENN

Antibody diversity

The Variation and Adaptive Expression of Antibodies. By George P. Smith. Pp. xii+219. (Harvard University: Cambridge, 1973.) \$12.

THE comparative analysis of amino acid sequences of immunoglobulins has been a major preoccupation of immunologists and molecular biologists for a number of years. The mass of data and the conclusions drawn from this have had a fundamental influence on present ideas on the genetic bases of antibody diversity and the evolution of protein structure. This book presents an exhaustive computer analysis of the data available to the author at the time in an attempt to define evolutionary relationships.

The book starts with a short survey

of fundamental concepts, intended for readers who are unfamiliar with immunology, and goes on to present tables of all the amino acid sequences that are the subject of subsequent analysis. Unfortunately, since the tables were completed a very considerable amount of important data has been published. In this respect the book is already out of date and does not include an interesting variety of sequences of human, mouse or rabbit κ chains. The analysis of heavy chains fares worst as the book does not adequately cover human μ , α and ϵ chains or mouse γ chains.

A very useful chapter is then devoted to the procedures used for the reconstruction of protein evolution. This provides the reader with a good introduction to the intricacies of the computer programs used. This is followed by an elaborate study of the evolution of C regions. The analysis, although merely confirming accepted ideas, does provide a very balanced view on the reliability of the proposed evolutionary patterns.

The following two chapters are devoted to an exposition of different theories on the origin of antibody diversity and the contrast between the expectations of each theory and the evolutionary patterns derived from the data. The exposition of the theoretical expectations is clear and detailed. The arguments in favour of the germ line theories are presented at their best. This is not the case in the presentation of the opposed arguments. (For instance, the critical discussion on linked parallel mutations (page 103) is only marginally touched on and does not include the possible requirements of tertiary structure.) This is to be expected since the author presents himself as a convinced germ liner.

Three appendices cover references for all the proteins listed, a discussion on allotypes and on ribosomal RNA genes as a model of multigene evolution.

Altogether this is a very readable book which transmits the intellectual excitement of the intricacies of the significance of every possible evolutionary clue. But the problem of antibody diversity is no longer the exclusive domain of protein chemists and the impact of the book is likely to be affected by the success of other approaches. Such new avenues are not properly presented. I was particularly struck by the author's remarks on DNA-RNA hybridisation (page 112): "Conclusive results from this type of experiment depend on the purification of immunoglobulin mRNA . . . a difficult technical feat". Alas, such a "technical feat" was being performed in several laboratories and in fact relevant papers were published at the same time as this book.

C. MILSTEIN

Change in New Guinea

The Chimbu: A Study in the New Guinea Highlands. By Paula Brown. Pp. ix+151. (International Library of Anthropology.) (Routledge and Kegan Paul: London, July 1973.) £3.25.

DR BROWN has, during a period of 15 years, observed the effects of social change in the population of the New Guinea Highlands. This group of people was first discovered by an Australian prospecting expedition in 1933 and many observations were made by administration officials, missionaries and an occasional anthropologist. These records were available to Dr Brown and with her own observations from 1958 onwards, she has been able to make a careful study of the effects of social changes. There is probably no other country in the world where it has been possible to observe profound changes which have occurred over a relatively short time. New Guinea has consequently become a haven for anthropologists but Dr Brown has made one of the most complete studies.

The Chimbu are usually regarded as among the most intelligent and energetic people in New Guinea and as Dr Brown describes, after an initial turbulent period, they seem to be adopting European culture with apparent enthusiasm. The introduction of medical services in recent years has reduced the infant mortality and this, in turn, has caused overpopulation. A few years ago the severe disease kwashiorkor, due to under-nutrition, was prevalent amongst Chimbu infants but this no longer occurs. Local government councils, the introduction of the internal combustion engine for transport and for agricultural purposes, the provision of schools, trading stores, cash crops, missions, European dress, roads, the development of law enforcement and political debating are all part of the new European culture. It is a fascinating story related accurately and entertainingly by Dr Brown.

The 19 chapters of the book are clearly divisible into three parts. The first describes the historic features of the life of the Chimbu people, the second is concerned with the dynamics of tribal life and the third describes many of the effects of change.

A biologist perhaps might be pardoned if he regrets that the biological changes, especially those affecting the growth and development and general health standards of infants and children in the area, were not studied at the same time as the social changes. It is known that such changes have occurred and their correlation with the cultural and social study of Dr Brown would have been interesting. An adjacent area

was the scene of the recent study during the International Biological Programme. Regrettably the latter did not include a study of the effects of social change such as that undertaken by Dr Brown in Chimbu. The problems of communication between different groups of research workers are often as great in New Guinea as in the more advanced countries, and opportunities are lost.

R. J. WALSH

Soviet geology

Geology of the U.S.S.R. By D. V. Nalivkin. Translated from the Russian by N. Rast. Edited by N. Rast and T. S. Westoll. Pp. xviii+855. (Oliver and Boyd (Longman): Edinburgh, November 1973.) £25.

APART from a very slim volume, published in translation in 1960, this is the first and only comprehensive work on the geology of the Soviet Union in English and so, whatever its quality, 855 pages of close type must be taken very seriously. But in relation to the immense geoscientific output from the Soviet Union, with so many multi-volume series on systematic aspects of Soviet geology, this work also is possibly unique in covering so large a field within a single volume.

It suffers a little from age. The interval between 1962 when published in the Soviet Union and late 1972 leaves a time of very great progress in Soviet geology unrecorded. But the value of the work to the English reader is to set the whole range of Soviet geology in perspective in a single framework, which in broad outline at least is hardly affected by recent work. So this volume serves as an introduction for western geologists as well as Soviet students to that vast territory.

The scheme of the book is distinctive, and as in so many general works in Russian science there is a rather explicit philosophy, reflecting scientific as well as political fashion (more than ten years old). In this case the philosophy is made to work throughout the volume. In short it is that geological history can best be understood in terms of geosynclinal development taken in a somewhat comprehensive, genetic and extensive way so as to include many aspects of tectonics including orogeny.

In consequence the book is classified thus: part 1, introduction; part 2, Precambrian geosynclines (Russian platform; Siberian platform; Western Siberian lowlands); part 3, Palaeozoic geosynclines (Uralian geosynclines; Western Arctic geosynclines; Argara geosynclines; Middle Asiatic geosynclines); and part 4, Meso-Cainozoic geosynclines (Mediterranean geosynclines; Pacific Ocean geosynclines).

There is indeed considerable attention paid to these controlling concepts both in the introduction with its historical review of Russian geology and a treatment of such principles, as well as in the translator's introduction. This treatment is indeed quite effective because geosynclines are strata, and the book adopts a thorough and explicit stratigraphical approach to tectonics, magmatism, and useful minerals in each chapter. This coherence is its strength in that a great amount of detail is well organised; it is also well referenced and well indexed.

Because such a unified approach is, with increasing knowledge and specialisation in the Soviet Union, likely to become less and less possible, this work will probably stand without competition as one of the classic regional descriptions, in the line of Heim's *Geologie der Schweiz*, David's *Geology of Australia*, and the recent multi-author Geological Survey work on Canada. Indeed national pride harnessed in this way is most welcome.

Professor Rast is an experienced translator and the work reads well in English. The many photographic illustrations give a sample of varied field conditions in that vast territory directed towards the student population about to explore it. There are a number of attractive sections but as with many Russian works the maps leave much to the imagination. The work is interspersed with innumerable fold-out correlation charts from which are seen, at a glance, both the regional variations and the contemporary Soviet view of stratigraphic classification.

W. B. HARLAND

Genetics practical

Practical Genetics. Edited by P. M. Sheppard. Pp. xii+337. (Blackwell Scientific: Oxford and London, 1973.) £8.50.

FIRST experiences of practical classes in biology are usually traumatic but rewarding for students and teachers alike, for what they observe for themselves often differs from what they were led to expect by plausible lecturers and the pedantry of textbook figures. This is particularly so in genetics practicals where the main technique of crossing and progeny analysis rarely provides the same goodness of fit to expectation which blessed Mendel's results. A working knowledge of statistics goes hand in hand with practical work in genetics and this, coupled with problems of obtaining suitable material, has given rise to the misleading impression that successful practicals are too difficult to organise.

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and the final year of degree courses will be grateful to Professor Sheppard and the other contributors for producing this excellent hybrid between text book and practical manual. The seven chapters include full descriptions of the techniques of cytogenetics and the culture methods for a wide range of organisms with detailed practical guides to exercises in basic genetics using *Drosophila*, fungi, bacteria and bacteriophage. Other chapters outline exercises in population, ecological and quantitative genetics and provide clearer accounts of biometrical genetics and elementary statistics than are found in most textbooks. Perhaps the chapter of most general value is the one concerned with higher organisms where a mine of information on the use and availability of teaching material emphasises that practicals in genetics need not be restricted to the few organisms commonly used in research.

Some of the suggested exercises can each be carried out by individuals in a single practical session, whereas others require the cooperative efforts of a number of students over a full term. All of the authors have provided a useful list of altruistic university departments and commercial suppliers that can provide suitable materials.

The editing is lenient, allowing some useful repetition, but the legend to plate 4.7 underlines the role of practicals in encouraging students in critical observation: those who follow the instructions and prepare slides of meiosis in locust species, might question whether the plate depicts diakinesis in *Schistocerca gregaria* or diplotene in some other species. JOHN GIBSON

Protein in stereo

Atlas of Molecular Structures in Biology. 1, Ribonuclease-S. Prepared by F. M. Richards and H. W. Wyckoff; edited by D. C. Phillips and F. M. Richards; figured by J. L. Mouning and J. W. Schilling. Pp ix+75. (Clarendon: Oxford; Oxford University; London, 1973.) £3.50.

PRESENTING the structure of a protein molecule is perhaps as immense a problem as solving the structure. In this volume on ribonuclease-S the editors and authors present a workable format for this task. It is the intention of the series to present structure unencumbered by interpretation. A short description of what the enzyme does, giving appropriate references to more detailed reviews and primary source material, starts the pleasantly brief text. After all, it is the pictures which are important in this book. Following is a series of tables and figures giving such items as physical properties, amino acid

sequences, Cartesian coordinates, hydrogen and ionic bonds, and various contact and angular properties of the chain. Some of the measured coordinates and angles are presented in several different forms, thus eliminating some conversion by the reader and making the work more usable.

The heart of the volume is the computer-generated drawings of the molecule. Forty-three red-green stereo drawings depict the main chain and all non-hydrogen side chain atoms from three mutually perpendicular directions. Four of these are specially devoted to the environment around the UpcA inhibitor complex. Many of the others are taken in ordered slices through the molecule. The publishers did an excellent job in the alignment of the stereo pairs but there was a slight shadow effect caused by an incorrect match of the green ink with the green filter in the stereo glasses provided. This effect became more pronounced as one moved closer to the diagram to read the small residue labels. From normal viewing distance the three-dimensional effect was quite good.

The last section contains the electron density maps for the apo-protein. This section should give the non-crystallographer the opportunity to see how a complete electron density map looks. The electron density of each section of the map is printed in green, and superimposed on it are all the atoms involved in that section with their Van der Waals radii drawn in red. By closing one eye and using the stereo glasses, one can see either the map or model. This section is excellent in its concept and execution. Unfortunately there is no index as to which sections on the map a residue will appear in, so the coordinate list must be used.

In general, the volume provides an excellent source for the structure of ribonuclease-S. Distances between residues of interest can easily be calculated from the coordinate lists and environment relationships can be found by consulting the many stereo diagrams. The major fault of the series is not in this volume, but perhaps in future volumes. Ribonuclease-S is approximately one-fifteenth the size of the larger multi-component proteins now being studied. The inclusion of the necessary diagrams to fully depict the subunit structure and the important subunit-interactions of one of these large proteins would not only make the volumes larger, but more expensive to produce and to purchase. Unfortunately, there seems to be no simple solution to the problem of disseminating all the structural information of a molecule as large as a protein, and this volume presents a good compromise solution. STEVEN J. STEINDEL

Muscle and silk

Conformation in Fibrous Proteins and Related Synthetic Polypeptides. By R. D. B. Fraser and T. P. MacRae. Pp. xviii+628. (Molecular Biology: An International Series of Monographs and Textbooks.) (Academic: New York and London, December 1973.) \$45; £19.50.

THE fibrous proteins form an important class among the complex structures found in living organisms, and the investigation of their structure poses special problems because they cannot, in general, be obtained in the form of single crystals suitable for X-ray investigation. The first part of this book describes the major techniques in current use and their application to the problems. They include X-ray, electron and optical diffraction, electron microscopy, infrared spectroscopy (using polarised radiation) and the important if less direct techniques based on the use of known atomic dimensions and interaction energies which may be described as model building. There is also a chapter on methods for obtaining the optimum structure and another on several techniques of more limited application.

The second part describes in considerable detail the several well-characterised conformations found in synthetic polypeptides (α -helix, β -conformation and so on) which have been basic to an understanding of the structure of fibrous proteins, and there are separate chapters on the silks (of which many kinds are known), collagens, muscle proteins and the keratins. There is a chapter on fibrin, fibrinogen, flagellin, elastin and resilin.

The writing is lucid and critical. The many line diagrams are excellent and there is a good selection of half-tone reproductions, chiefly of diffraction patterns and electron micrographs. The list of references is very comprehensive and it is evident that care has been taken with the indexes. The authors (who have made major contributions to our understanding of fibrous protein structure) are to be congratulated on the publication of such a thorough and useful work.

A. ELLIOTT

Drifting in the Pacific

The Settlement of Polynesia: A Computer Simulation. By M. Levison, R. Gerard Ward and J. W. Webb. Pp. vi+137. (University of Minnesota Press: Minneapolis, 1973.) £5.50.

THE question of how the scattered islands of Polynesia were settled during a 2,000-year period ending about a thousand years ago has been a perplexing one for European colonisers

whose own methods of navigation only allowed them to arrive there less than 300 years ago. One rather loud voice in the debate in recent times has been that of Andrew Sharp. He simply applied to the problem his hard-headed common sense, Occam's razor, and a due cynicism about far-fetched or fantastical tales of intrepid ancient Polynesian seamen with sophisticated navigational skills. After all, Polynesians were both illiterate and living in the Stone Age. Sharp's thesis is that island discovery took place by un navigated one-way voyages either of exile, or accidental drift, and that there is no reason to suppose that deliberately navigated journeys of exploration or long voyages with return to the starting place occurred. In the preface to his 1964 book, Sharp states, "It is six years since my previous book . . . During those years I have gathered upwards of a hundred published notices . . . and have been involved in 2,191 oral discussions of the book's themes. I have yet to hear of a fact or read an argument which impugns the basic contentions of the former book". The present book by Levison, Ward and Webb, contains both facts and arguments which do impugn Sharp's contentions.

What Levison, Ward and Webb have done is to take the recorded data of frequencies of different winds and currents throughout the Pacific, and simulate by computer one-way drift voyages from a variety of starting points.

All the assumptions of the simulation seem remarkably sensible. On each day a particular wind and a current direction are chosen randomly from the available recorded frequencies of winds and currents for the particular month in the particular $5^\circ \times 5^\circ$ (approximately 300 nautical mile) square, and the vessel is moved an appropriate distance and direction. A craft makes a landfall if it passes within 10 miles of a low island or 20 miles of higher ones. There is a sigmoid curve of increasing tendency of a voyage to end due to factors like death of the crew (50% of voyages terminate in this way by 10 weeks). But there is also a 0.5 probability of foundering if a gale of force 9 or above occurs.

The authors have simulated more than 100,000 drift voyages, and 8,000 deliberately guided ones. The drift voyages included a main series of 46,000 from 61 starting points starting every day of the year, 11,000 under shifts of climatic conditions of the kind that might have occurred in the last 2,000 years or so, 32,000 starting from specially advantageous points at specific months of the year, and 10,000 in reverse from destinations towards possible starting points.

Their conclusion was that drifting produced a zero probability of reaching either Hawaii or Easter Island from any inhabited part of Polynesia, and less than 0.1% chance of reaching New Zealand. Thus drifting alone does not seem to be able to account for the colonisation. Among other things they also show that Kon Tiki would never have reached Easter Island, or anywhere else in Polynesia unless it had been very deliberately steered westward (which it was). Indeed, in simulated voyages with an attempt to hold a particular course, and with a limitation of only being able to lay as close as 90° to the wind, the chances of reaching the outlying parts of Polynesia become quite reasonable.

Levison *et al.* are judicious on the subject of relevant archaeological data and the various theories that have been put forward. They also are scrupulous about the technical part of their simulation. Both a complete listing of their ALGOL program and a large selection of their results in the form of maps of scattered destinations of voyages from particular starting places appear in appendices which occupy about half the book. But the non-technical should not be deterred. The text though detailed is well written, and the whole is a substantial contribution. Put together with the recent publications by Gladwin and Lewis of sophisticated navigational techniques still practiced in the Caroline Islands, this work finally disposes of the idea that Polynesians were savages who just happened to have been blown to accidental discovery of the vast reaches of the Pacific which they came to inhabit.

KEITH OATLEY

Relativity with reprints

General Theory of Relativity. By C. W. Kilmister. Pp. ix+365. (Commonwealth and International Library: Selected Readings in Physics.) (Pergamon: Oxford and New York, November 1973.) £3.50 boards; £2 paper.

ACCORDING to the publisher's note on the back cover, this book meets "the increasing need for an undergraduate text on the general theory of relativity". It will be an unusual undergraduate who absorbs more than a portion of it but there is a need for a textbook for all students with ambitions to research into relativity, cosmology and astrophysics (and pure differential geometry). This should ideally not only present the facts about relativity they need, but also assist the transition from didactically helpful lectures to the scientific literature.

This book is important because it has both these aims. Before venturing

to carp at unimportant idiosyncracies I should say that it is wholly recommended to such students because it largely fulfills them. The first hundred pages contain the author's exposition of the principle of equivalence, of Einstein's theory and of more recent developments. The rest of the book consists of eleven reprinted papers.

The author's account of the motivation for the theory is largely from the equivalence principle. Mach's principle is dealt with summarily; Einstein is said (page 12) to have hoped that his theory would incorporate it but the grounds for this hope are not made clear. Eddington's demonstration that relativity diminishes the number of constraints that "laws of nature" impose on the world is scarcely mentioned though for many students it is the most stimulating feature of the elementary part of the theory. Cosmology is dealt with quite briefly; the Robertson-Walker models are not derived and the book is not advanced enough for global properties or singularity theorems. Gravitational radiation is taken no further than the classic 1962 paper of Bondi, Burg and Metzner which is reprinted with explanation in the text; and the spinor calculus no further than Penrose's 1960 paper reprinted without.

The book is therefore not quite an introduction to current research, but an advanced textbook, and a very good one.

P. E. ROE

Teaching mathematics

Developments in Mathematical Education. Edited by A. G. Howson. Pp. ix+318. Proceedings of the Second International Congress on Mathematical Education, Exeter, UK, August 1972.) (Cambridge University: London, 1973.) £4.80; \$14.50.

THE conference reported by this book consisted of thirty-eight working groups which dealt with the subject, on a broad basis, from kindergarten to the university.

The report appears in three parts. The first consists of a survey of the congress and covers a wide range of topics, including the psychology of mathematics learning; mathematics and language; mathematics at primary and secondary level and its link with other subjects, applications, history and assessment of mathematics; professional training of teachers and the use of teaching aids; and mathematics in developing countries.

The second part of the report consists of invited papers whose authors include G. Pólya, J. Piaget and Sir James Lighthill. There is also a paper by S. L. Sobolev on "Some Questions

of Mathematical Education in the U.S.S.R.". The third part consists of a selection of congress papers. These cover problem solving; intuition, structure and heuristic methods; geometry as a gateway to mathematics; and the work of Piaget in the training of students to teach primary mathematics.

My impression of the present state mathematical education is that too much is going on at the same time on too many fronts. Confusion is evident in the clash of differing philosophies, and it will probably be a matter of decades before a settled place is found for mathematics as a field of human endeavour. There seems to be far too much experiment, much of which is badly planned, or, worse still, introduced on impulse. But mathematical concepts, as a way of thinking, are recognised as being of greater importance than in any past period of history. Mathematics is no longer a matter only of number and numeracy. Structure, including concepts and their relationships, is now consciously identified as a fundamental aspect of human thinking. One of the main problems of mathematical education in the coming years will be to determine the place of structure in the thinking and behaviour of the mass of mankind.

L. S. GODDARD

Air and ocean

The Physics of Air-Sea Interaction. By S. A. Kitaigorodskii. Translated from the Russian. Pp.v+237. (Israel Program for Scientific Translations: Jerusalem, 1973.) n.p.

THIS book will undoubtedly become an essential work for the ever increasing circle of physicists, meteorologists and oceanographers for whom the interaction of the air with sea has become an important problem. Although there are omissions it will provide an up-to-date text upon which to build some of the newest theories of air-sea interaction.

The book is written in three parts. The first part, "Dynamics of the Marine Surface Mixing Layer of the Atmosphere", is devoted to the physics of the layer of the atmosphere immediately adjacent to the water surface. From the starting point of the Monin-Obukhov form for the generalisation of a logarithmic boundary layer to a temperature-stratified medium, the author examines the usual turbulent fluxes of momentum, heat and water vapour before continuing with a study of the aerodynamic drag of the sea surface and its relation to the characteristics of the turbulent atmosphere and of wind-generated waves. Several chapters are devoted to the analysis of results from field measurements of atmospheric

turbulence over waves in the Mediterranean and show some of the procedures used for evaluating the results from this complex problem. The last chapter in part 1 is concerned with the possible influence of high humidity on the vertical density stratification over oceans and collates and analyses data from many sources on gradient measurements and standard seaborne observations.

Part 2, "Wind Waves in Deep Sea" is a description of some fairly general similarity hypotheses concerning wind waves, and of tests of these on experimental data. The author attempts to reduce the complexity of the problem by obtaining some universal dimensionless parameters to describe the relationship between the turbulent wind and the spectrum of the wind waves. The approach and content is very different to that found in Kinsman's *Wind Waves* and these two texts complement each other well. Again there is much use made of experimental data with prominence given to the Soviet Mediterranean expeditions in 1965. Particularly interesting are the results from a wide-band string wave recorder used to obtain extensive measurements of open-sea wave characteristics in the high frequency region (up to 7.5 Hz). No mention is made, other than a statement confirming its omission, of the non-linear interaction between components in the wave spectrum.

In the third part, "Dynamics of Vertical Mixing Processes in the Upper Ocean", the author turns from his extensive use of experimental data and considers some of the many physical hypotheses underlying the characteristics of sea turbulence and the ways in which they effect the vertical mixing processes. Several models are used to make predictions about the energy balance of dynamic small scale turbulence in the wind mixing layer. The final chapter considers the seasonal variations in the active layer of the ocean. It seems a little out of place in a book concerned mainly with the physics of small scale interactions. Perhaps the author is right at this point to expand the field of view and to introduce some long term variations.

The text is also valuable for the attention it pays to some of the less well known expedition results of the Institutes of Atmospheric Physics and Oceanology of the Academy of Sciences of the Soviet Union and for the comparisons made between these data and those of the more usually quoted sources. Overall, an excellent book; my only criticisms are the minute size of type used for the equations and the confusing choice of symbols on a few diagrams.

C. E. VINCENT

science on television

Human face of Watson and Crick

by Peter Newmark

Back in the balmy days of the early 1950s, before the national press was heavy with doom and the scientific press with molecular biology, small groups of scientists in London, Cambridge and California were working away on the academic problem of the structure of DNA. And then it happened. The day after Mr Churchill became Sir Winston, Francis Crick and James Watson published their proposed structure for DNA in these pages and "The Race for the Double Helix" was over. The Horizon programme of that title dealt with the last few months leading up to this discovery.

Treading much the same ground as, and in a similar vein to, Watson's book *The Double Helix*, the programme dealt with the people involved much more than with the science. Indeed the only real bit of experimentation shown, R. G. Gosling trying to stretch a DNA fibre, failed (intentionally?) in front of the camera. What was shown, and very successfully, was the way in which the course of the race was affected by the personalities involved.

The story was narrated by Sir Michael Swann and divided into segments each introduced, silent movie style, with a still caption—such as "The Race Is On". But anyone expecting matching melodrama, villains and chivalrous heroes would have been disappointed. The characters were no larger or better behaved than life. There were Maurice Wilkins and his group studiously improving their X-ray data and moving towards the answer, Linus Pauling applying his expertise to other X-ray photographs and Watson and Crick building models *ad infinitum*.

All these people and more appeared to tell their tale. Of the main characters only Rosalind Franklin is no longer alive. It was her involvement that provided the most human touch to the whole story. Employed by Wilkins for her knowledge of crystallography, it soon became evident that the two were incompatible. This, or so it was related in the programme, resulted in a degree of isolation for Franklin that probably cost her the first place in the race. Nor were relations between her and Watson much better. He needed her data but

could offer little in return. Meetings were tense and even degenerated to fisticuffs on one occasion. Pauling was equally human. Although expecting to discover a double helix, he made a crucial misinterpretation of some data and opted for a triple helix. His son passed a pre-publication copy of the paper describing this to Watson and Crick. Their disbelief in the proposed structure acted as a spur for their own activities.

From their own account, surely a bit exaggerated by the passing of years, theirs was casual labour. Between coffee and tennis, Watson would play with bits of cardboard whilst Crick, trying to complete a mature doctoral thesis, would occasionally add a word of advice. Whatever the truth, and Watson did at one stage admit to working hard, the base pairing was realised and the full structure immediately followed.

Crick has recently written that in their classic paper "the structure is produced like a rabbit out of a hat, with no indication as to how we arrived at it." The nice thing about the programme was that it did show how, and in human, rather than scientific, terms. It was an excellent exercise in demonstrating the foibles and failings of scientists and acted as a splendid counterbalance to the reverent attitudes to which even Horizon is often prone. Nevertheless it was made quite clear that some three months before Mount Everest was first climbed, a scientific discovery of equal magnitude had been achieved.

The Valley of Paradise

by Allan Piper

LIKE Liverpool versus Leeds, and Royal Weddings, Vilcabamba—the "Valley of Paradise"—and its extraordinary inhabitants might have been made for television. In a beautiful, sun-filled bowl, 6,000 feet up in the Ecuadorian Andes, a community of gentle peasants farm the fertile land. Amazingly, in spite of a daily consumption of 40 cigarettes, four cups of locally distilled, 110° proof rum, and the occasional guinea pig, many of these strange people live to ages of more than 120 yr. And as if that were not enough to ensure a captive audience, there is a hint that it could be attributable to a healthy

sex life. But disappointingly, and uncharacteristically, Granada's "World in Action" team never quite made the best of what was bound to be a good documentary.

Arriving just ahead of the new road through the mountains, and the inevitable tourists, the camera crews have been beaten to Vilcabamba only by the scientists.

Many of their theories are predictable: "The air is so pure" and so on. Other theories are more unorthodox and intriguing. It has been suggested that centuries of close inbreeding have isolated a genetic abnormality, or more remarkable, that after a lifetime of climbing steep slopes and working the land, the calf muscles of these people have become so powerful that they operate as a secondary heart. But the inhabitants of Vilcabamba pay a price for their longevity: half of the children of the valley die before they are 10.

In spite of its unusual content, however, the documentary, (screened on July 8) lacked a sense of coherence. It was never cogent or compelling. Perhaps that was because "World in Action" tried to do too much in too little time. As a result, the programme was often vague and contradictory. We were told that, for example, all the peasants are vegetarian, and also that some eat donkey meat.

We were also presented with various nuggets of hollow information: There are two rivers flowing through Vilcabamba. One is warm, the other is cold. Ho hum. And we were treated on the basis of slender evidence to the thesis that an old woman might be so old because of her healthy attitude towards sex. "Did you have many lovers?" she was asked. Well, she was married once but her husband died a long time ago; just as well really, he used to be a devil—always beating her. Nearly killed her once. Eeee.

Miguel Carpio, 132 years old, tall and bronzed with long black hair and a snow white beard, retired from a life of farming at 125. Healthy and in command of all his mental faculties, he sits upright and impassive as the young intruders from the outside world try to come to terms with his only obvious handicap. "Do you", bawls the reporter, an impolite six inches from Miguel's left ear, "still like women?" Obviously. Miguel is going deaf. Probably, it doesn't bother him very much: at his age he's undoubtedly heard it all before.

Science broadcasting statistics

The proportion of BBC radio time spent on science has never been lower, in spite of the fact that the proportion of time devoted to talks and discussions has been increasing over the past ten years. Yet television has shown that there is a market for science, with ten million viewers watching *The Burke Special*.

James Friday, an archivist on the staff of the Royal Institution has been collecting statistics on the BBC's use of programme time. In 1923 Reith, with his brief to educate, inform and entertain devoted $\frac{1}{2}\%$ of radio time to

scientific programmes. Subsequently the emphasis shifted heavily to entertainment and science was relegated to schools programmes. In 1943 with science up to about 1%, the British Association, whose general secretary was then Richard Gregory, editor of *Nature*, sent a delegation to the BBC to suggest improvements in science broadcasting. The delegation recommended that the BBC should appoint a scientific advisory group and a scientific programme organiser. The BBC rejected both proposals. When similar proposals were made twenty years later by the Pilkington Commission they were accepted in part: a Science Consultative Group was appointed in 1964. But, its influence seems to have been negligible.

Television science has clearly not suffered from the BBC's dislike of having scientists in its midst. But radio producers seem to have become progressively more intimidated by the subject: science at present occupies $\frac{1}{3}\%$ of BBC radio time. With television science included, the proportion rises to slightly over 1%—little more than in 1943. Yet surely science impinges on most peoples' lives considerably more than it did in 1943? The BBC—and scientists—should consider, said Friday in a recent RI lecture, how they can best transmit science and how many hours a week they need to do it before they are overtaken by the report of the Annan Commission and the timidity of the programme producers.

Announcements

Appointments

Roy Gibson has been appointed Acting Director General of The Council of the European Space Research Organisation.

Erratum

In the article 'Nuclear fission as a general source of energy' by L. R. Shepherd (*Nature*, **249**, 717; 1974) the two figures were inadvertently printed above the wrong figure legends. The legends themselves are correct and in the right places.

International meetings

September 2–6, **International Symposium on Approaches to the Early Detection of Chemical Toxicity**, Guildford (Mrs J. King, Department of Biochemistry, University of Surrey, Guildford GU2 5XH).

October, **International Symposium on Andean and Antarctic Volcanology Problems**, Chile (Professor O. Gonzales-Ferran, Department of Geology, University of Chile, Casilla 13518, Correo 21, Santiago, Chile).

October 6–11 **Symposium of the International Society for Photogrammetry**, Banff, Canada (Dr L. Sayn-Wittgenstein, Canadian Forestry Service, Ottawa, Canada).

October 7–11, **First World Congress of Genetics Applied to Animal Production**, Madrid (Professor Dr Luis de Cuenca, Fac de Vet, University of Madrid, Ciudad University, Madrid, Spain).

October 10–12, **International Symposium on Thermogenesis and Depressed Metabolism**, Czechoslovakia (J. Meisner, MD, CSc, General Secretary, Faculty of Natural Sciences, Vinicna 7, 120 00 Praha 2, Czechoslovakia).

October 13–16, **Symposium on Temperature Regulation**, Israel (The Secretariat, POB 983, Jerusalem, Israel).

October 13–16, **Symposium on the Mechanisms of Synaptic Action**, Israel (The Secretariat, POB 983, Jerusalem, Israel).

October 13–17, **Symposium on Environmental Physiology** (The Secretariat, POB 983, Jerusalem, Israel).

October 13–19, **Fifth World Congress of Gastroenterology**, Mexico (V Congreso Mundial de Gastroenterología, Avenida Veracruz 93, Mexico 11 DF, Mexico).

October 14–18, **International Symposium on the Regulation of Growth and Differentiated Functions in Eukaryote Cells**, New Delhi (Professor G. P. Talwar, Department of Biochemistry, All India Institute of Medical Sciences, New Delhi, 11016, India).

October 20–22, **Sixth Meeting of the International Association for the Study of the Liver**, Acapulco (VI Reunion de la Asociacion Internacional para el Estudio del Higado, Avenida Veracruz 93, Mexico 11 DF, Mexico).

October 21–23, **3rd Conference on Application of Small Accelerators**, Texas (Dr Jerome L. Duggan, Department of Physics, N. Texas State University, Denton Texas 76203).

October 21–25, **Symposium of the International Atomic Energy Agency on the Thermodynamics of Nuclear Materials**, Vienna (International Atomic Energy Agency, PO Box 590, A-1011 Vienna, Austria).

October 21–25, **Second Conference of the Condensed Matter Division of the European Physical Society**, Budapest (EPS Condensed Matter Division, Conference Office, Research Institute for Technical Physics, 1325 Budapest, PO Box 76, Hungary).

Reports and Publications

Great Britain

Department of Trade Industry, Warren Spring Laboratory—Annual Review, 1972/1973. Pp. 32. (Stevenage, Herts: Warren Spring Laboratory, 1974.)

Bulletin of the British Museum (Natural History). Historical Series, Vol. 4, No. 5: Sir Joseph Banks and the Plant Collection from Kew Sent to the Empress Catherine II of Russia, 1795. By H. B. Carter. Pp. 281–385 + 4 plates. (London: British Museum (Natural History), 1974.) £5.45.

British Museum (Natural History). Entomology Leaflets. No. 1: Insects. Pp. 6. 5p. No. 2: Insects and Man. Pp. 12. 9p. No. 3: Aquatic Insects. Pp. 6. 5p. No. 4: Insect Flight. Pp. 6. 5p. No. 5: Butterflies and Moths. Pp. 6. 5p. Zoology Leaflet No. 8: The Domestic Dog. Pp. 6. 5p. (London: British Museum (Natural History), 1974.)

Microbiological Research Establishment—Abstracts of Work published in 1973. Pp. 19. (Porton Down, Salisbury: Microbiological Research Establishment, 1974.)

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 267, No. 889: Late Quaternary History of Vegetation and Climate of the Rajasthan Desert, India. By G. Singer, R. D. Joshi, S. K. Chopra and A. B. Singh. Pp. 467–501. (London: The Royal Society, 1974.)

Other Countries

Australia: Commonwealth Scientific and Industrial Research Organization. Division of Soils Technical Paper No. 21: The Computation of Optimal Rates of Application of Fertilizers from Quadratic Response Functions. By J. D. Colwell. Pp. 15. (Melbourne: CSIRO, 1974.)

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Bulletin 224: Carboniferous and Permian Stratigraphy of Axel Island and Western Ellesmere Island, Canadian Arctic Archipelago. By R. Thorsteinsson. Pp. 115 (27 plates). \$6. Memoir 368: Surficial Geology of Avalon Peninsula, Newfoundland. By E. P. Henderson. Pp. 121. \$4. Paper 72–51: Surficial Geology of the Kananaskis Research Forest and Marmot Creek Vasin Region of Alberta. By A. MacS. Stakler. Pp. 25. \$2. (Ottawa: Information Canada, 1972, 1973 and 1974.)

World Health Organization. Technical Report Series. No. 541: Disposal of Community Wastewater. Report of a WHO Expert Committee. Pp. 72. Sw. fr. 6. No. 542: WHO Expert Committee on Filariasis—Third Report. Pp. 54. Sw. fr. 5. (Geneva: WHO; London: HMSO, 1974.)

CERN—European Organization for Nuclear Research. CERN 74–8: Topical Meeting on Intermediate Energy Physics, Lyceum Alpinum, Zuoz, Engadin, Switzerland, 4–13 April 1973—Proceedings. Pp. ix + 220. (Geneva: CERN, 1974.)

Smithsonian Contributions to Zoology, No. 164: Synopsis of the Families and Genera of Crayfishes (Crustacea: Decapoda). By Horton H. Hobbs, Jr. Pp. iii + 32. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) 80 cents.

Institut für Atomenergi, Kjeller. Kjeller Report 149: The Electrochemistry of Uniform Corrosion and Pitting of Aluminium. By K. Videm. Pp. 85. (Kjeller, Norway: Institut für Atomenergi, 1974.)

Bulletin of the Fisheries Research Board of Canada, No. 186: The Capelin (*Mallotus villosus*) Biology, Distribution, Exploitation, Utilisation, and Composition. By P. M. Jangaard. Pp. x + 70. (Ottawa: Information Canada, 1974.) \$3.60.